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SOIL SCIENCE

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THE TEMPERATURE CORRECTION IN THE HYDROMETER METHOD OF MECHANICAL ANALYSIS OF SOILS

CHARLES RICHTER

Hawaii Agricultural Experiment Station

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The employment of the hydrometer for the measurement of soil-suspension concentrations in the course of mechanical analysis has become quite widespread since its proposal by Bouyoucos in 1927. The method has been found valuable by many investigators for routine analysis in soil surveys and for soil classifications.

The method, which is said to be based on heat of wetting determinations, has been checked by several investigators against other methods such as the pipette method and the water-vapor adsorption method. It appears that the hydrometer method agrees fairly well with the pipette method, but not so well with the water-vapor adsorption method in the case of soils of high organic matter content. This latter fact of course does not discount the value of the hydrometer method; it simply means that with certain soils it seems inadvisable to attempt to translate physical properties into particle size or vice versa.

The hydrometer method is too well known to be described here again in detail, and for this reason only a very short resumé is given.

An aliquot of soil (25, 50, 75, or 100 gm.) is dispersed, in a specially constructed metal cup fitted with buffers, by an electric mixer for a required length of time with the addition of 5 cc. *N* KOH solution. After the dispersion has been completed the contents are transferred to a glass cylinder, made up to a definite volume with distilled water, shaken, the hydrometer placed into the soil suspension, and the mixture allowed to settle. After 15 minutes the hydrometer readings which give the amount of soil in grams left in suspension, are taken. This is considered as the colloidal fraction. The time for complete dispersion as recommended by Bouyoucos is 9 minutes, which is probably sufficient for many mainland soils but very insufficient for heavy lateritic or organic soils. For most Hawaiian soils at least 30 minutes dispersion is required, in certain cases 60 minutes. In this investigation 30 minutes dispersion was used in every case. The water used was 1,000 cc. plus as many cubic centimeters more as there were grams of soil. The length of time required for settling assumes a uniform specific gravity of about 2.65 for soils; for a more exact and detailed mechanical analysis the settling time must be calculated from the specific gravity of soils, the temperature of the mixture, and the height of the column of suspension. Since the hydrometer is calibrated to give correct readings at 67°F., under or above this temperature a necessary correction is made, i.e., 0.35 is added to the reading for each degree above 67°F., and subtracted for each degree below 67°F. The corrected reading divided by the weight of soil used gives the percentage of fraction in question.

Although the practical usefulness of the hydrometer method is recognized, it is desirable to know the degree of accuracy of which it is capable at low range as well as at high range. Is the same correction to be applied to the readings at all concentrations and at all temperatures? It is evident from fundamental considerations that the temperature correction of 0.35 for each degree cannot hold true for the whole range of the hydrometer and that the error introduced becomes greater as the concentration becomes lower. Moreover, it may be anticipated that at any one concentration the temperature correction at different temperatures will not be a straight line function but a curve.

TABLE 1

*Relation of concentration of soil suspension to the error involved in the application of the temperature correction of ± 0.35 factor to the hydrometer reading in case of a typical Hawaiian clay**

AMOUNT SOIL USED	HYDROMETER READING AT 67°F.	HYDROMETER READING AT 87°F.	COLLOIDS ISOLATED	COLLOIDS PER GM. SOIL CALCULATED FROM READING AT 67°F.	COLLOIDS CALCULATED FROM READING AT 87°F. USING 0.35 FACTOR	COLLOIDS PER GM. SOIL CALCULATED FROM WEIGHT OF COLLOIDS	ERROR INVOLVED DUE TO TEMPERATURE CORRECTION	COMPUTED CORRECTIONS TO GIVE APPROXIMATELY CORRECT RESULT
(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
gm.			*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	
100	63.0	56.0	65.7	63.0	63.0	65.7	0.0	0.35
80	50.5	45.0	51.3	63.1	65.6	64.1	4.1	0.27
60	37.5	33.0	38.5	62.5	66.7	64.1	5.9	0.24
40	24.5	21.0	24.9	61.2	68.8	62.2	9.2	0.21
20	12.0	9.0	11.4	60.0	80.0	57.0	27.0	0.18
10	6.0	3.5	6.1	60.0	105.0	61.0	66.7	0.14
5	3.0	0.5	3.0	60.0	150.0	60.0	138.1	0.13
Average.....						62.9†	.....	.....

* Figures expressed in terms of oven-dry soil.

† Omitting fifth number, for 20 gm. of soil.

In the following experiments, the effect of concentration and temperature of the soil suspension on the accuracy of the hydrometer method has been investigated, both mainland and Hawaiian soils, high in clay fraction, being used.

The soils used in this investigation were as follows:

1. Light grayish-brown river-bottom clay from Arlington farm, Virginia. Specific gravity, 2.62; organic matter, 5.72 per cent; pH, 5.30.
2. Light-brown Pacific Coastal soil from Bellingham, Wash. Specific gravity, 2.54; organic matter, 3.21 per cent; pH, 5.70.
3. Light-brown clay from Lanikai, Oahu, Hawaii. Specific gravity, 2.83; organic matter, 3.94 per cent; pH, 8.23.
4. Yellowish-brown clay from Hakalau, Hawaii. Specific gravity, 2.84; organic matter, 9.07 per cent; pH, 6.50.
5. Brownish-red clay from Maui, Hawaii. Specific gravity, 2.96; organic matter, 3.27 per cent; pH, 7.60.

Table 1 shows the relation of the soil-suspension concentration to the error involved in the application of the temperature correction of plus-minus 0.35 factor to the hydrometer reading in the case of a typical Hawaiian clay (soil 5). Various amounts of soil (column A) in simple multiples (5, 10, 20, 40, 60, 80, 100 gm.) were dispersed as described in the foregoing and brought to the required volume. The suspension was cooled down to 67°F., shaken and placed into a water-bath adjusted to 67°F., and allowed to settle for 15 minutes. At 15 minutes the hydrometer reading was taken (column B). The cylinder

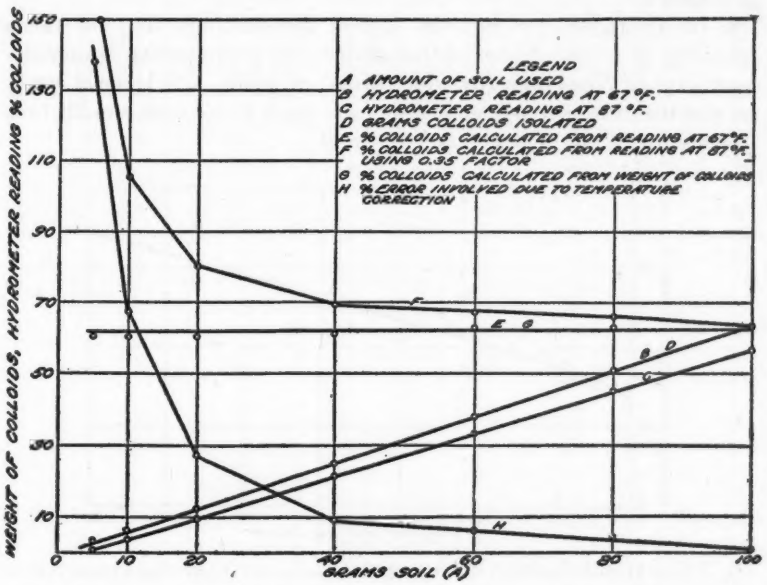

FIG. 1. RELATION OF CONCENTRATION OF SOIL SUSPENSION TO THE ERROR INVOLVED IN THE APPLICATION OF THE TEMPERATURE CORRECTION OF THE ± 0.35 FACTOR TO THE HYDROMETER READING

with its contents was placed into the thermostat for overnight at 87°F. The hydrometer reading was again taken after 15 minutes settling at 78°F. (column C), and the soil suspension immediately siphoned off to the point where the solid matter was settling. The suspended solid matter was isolated by evaporating to dryness on the water-bath. The obtained colloids were then dried at 125°C. for 18 hours and weighed (column D). Column H was obtained by subtracting 63 (the assumed correct value) from the value given in column F and dividing by 0.63. The last column was obtained by the following formula: $\frac{0.63A-C}{20}$. These data are arranged graphically in figure 1. As may be seen from the foregoing the agreement between the weight of isolated

colloids and the hydrometer reading at 67°F. is good, but that the error involved in calculating the percentage of colloids from the hydrometer reading at 87°F. becomes considerable at lower concentrations when the same temperature factor of plus-minus 0.35 for each degree F. is used throughout. In this set of figures it was assumed that the correct percentage of colloids is 63, i.e., the reading at 67°F. of the soil suspension when 100 gm. of soil was used. This figure agrees with the over-all average of the percentages of colloids calculated from the weight of colloids isolated for all concentrations (average of column G).

If the computed corrections at different concentrations (i.e., the values appearing in column I) are plotted against the corresponding hydrometer readings at 87°F. and connected with a smooth curve (fig. 2), it becomes apparent that the correction to be applied for each degree F. decreases steadily from the upper to the lower range of the hydrometer.

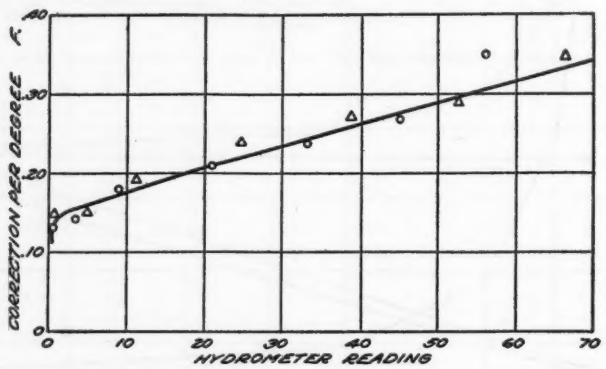

FIG. 2. CORRELATION BETWEEN HYDROMETER READING AND COMPUTED CORRECTION TO GIVE CONCORDANT RESULTS

It is to be noted that a very good parallelism obtains between the hydrometer reading at 67°F. and 87°F. when they are plotted against the concentrations.

In order to see the correlation between hydrometer reading and increasing temperatures at different concentrations, soil suspensions were made at three different concentrations (10, 50, and 100 gm. to a liter of water) with a number of soils, and their apparent density was successively determined by means of the hydrometer at increasing temperatures after 15 minutes settling. The soil suspension was cooled down to about 50°F., shaken, and allowed to settle for the required time, when the hydrometer and temperature readings were taken. This procedure was repeated several times at different temperatures as the soil suspension was warming up. After the last reading, which was taken at about room temperature (84–88°F.), the cylinder with its contents was placed

TABLE 2

Correlation between hydrometer reading and temperature at different concentrations, and the computed corrections

SOIL NUMBER	CONCENTRATION OF SOIL SUSPENSION								
	10 gm. per liter water			50 gm. per liter water			100 gm. per liter water		
	Hydrometer reading	Temperature	Computed correction per degree F.	Hydrometer reading	Temperature	Computed correction per degree F.	Hydrometer reading	Temperature	Computed correction per degree F.
		°F.			°F.			°F.	
1	5.5	49.5	−0.06	17.5	48.0	−0.11	31.5	46.0	−0.13
	5.2	56.0	−0.06	17.0	56.0	−0.14	31.0	52.0	−0.15
	5.0	60.0	−0.07	16.5	60.0	−0.14	30.5	58.0	−0.19
	4.5	67.0	*	16.0	63.5	−0.14	29.8	62.0	−0.20
	3.8	74.5	+0.09	15.5	66.5	*	29.2	64.5	−0.16
	3.0	81.5	+0.10	15.2	68.5	+0.13	28.5	67.5	*
	...	....	.....	14.5	74.0	+0.14	28.0	71.0	+0.20
	...	....	.....	13.5	79.5	+0.16	26.5	77.5	+0.22
	...	....	.....	11.5	87.0	+0.20	25.5	82.5	+0.21
	...	....	.....	....	....	.....	24.0	87.0	+0.24
2	6.0	50.0	−0.07	19.0	50.0	−0.16	33.5	52.0	−0.20
	5.8	56.0	−0.09	18.5	56.0	−0.20	32.5	58.0	−0.22
	5.5	62.5	−0.14	17.5	61.0	−0.20	31.2	63.5	−0.20
	4.7	68.0	*	16.5	66.0	−0.20	30.5	67.0	*
	3.5	75.0	+0.16	15.5	73.0	+0.16	30.0	69.0	+0.25
	2.5	81.5	+0.16	14.0	79.0	+0.19	29.0	73.0	+0.25
	1.5	87.5	+0.16	12.5	86.0	+0.20	27.5	79.0	+0.25
	...	....	.....	....	....	.....	26.0	84.0	+0.26
3	8.5	55.0	−0.10	33.0	52.0	−0.10	62.0	52.0	−0.19
	8.0	60.5	−0.11	32.5	58.0	−0.11	61.0	58.5	−0.21
	7.5	66.0	*	32.0	62.5	−0.11	60.0	63.0	−0.20
	6.5	73.0	+0.13	31.5	67.0	*	58.7	69.0	+0.25
	5.5	80.0	+0.14	30.5	74.0	+0.14	58.0	72.5	+0.22
	4.3	86.0	+0.16	29.0	80.0	+0.19	56.5	78.0	+0.25
	...	....	.....	27.0	86.0	+0.24	55.3	82.0	+0.26
	...	....	.....	....	....	.....	54.7	84.5	+0.26
4	8.0	51.0	−0.08	32.0	51.0	−0.11	62.5	53.0	−0.13
	7.5	59.0	−0.10	31.2	59.0	−0.11	61.7	60.0	−0.14
	7.0	65.0	−0.15	30.5	66.0	−0.20	61.0	66.0	*
	5.5	75.0	+0.15	28.5	76.0	+0.20	58.5	76.0	+0.24
	3.0	89.0	+0.17	26.0	85.0	+0.24	56.5	83.0	+0.26
5	8.0	55.5	−0.10	35.5	51.0	−0.16	64.5	50.0	−0.17
	7.5	62.5	−0.13	34.3	59.0	−0.16	62.7	62.0	−0.22
	7.0	66.5	*	33.5	65.0	−0.25	61.5	66.0	*
	6.0	74.0	+0.13	32.5	69.0	+0.25	60.0	73.0	+0.27
	4.5	80.5	+0.18	31.5	73.0	+0.25	58.5	80.0	+0.24
	3.0	86.0	+0.21	30.0	80.0	+0.23	57.0	86.0	+0.24
	...	....	.....	27.0	91.0	+0.25	....	....	.....

The weight of isolated colloids for the above soils were as follows for the 10, 50, 100 gm. per liter concentrations respectively: Soil 1, 3.3, 13.55, 31.2 gm.; soil 2, 3.2, 14.5, 33.1 gm.; soil 3, 6.1, 31.3, 66.3 gm.; soil 4, 5.7, 27.9, 57.7 gm.; soil 5, * * * (not determined).

into a thermostat and was allowed to come to a constant temperature (86°F.), when it was shaken up and allowed to settle for 15 minutes. The suspension was then siphoned off to the point where the solid matter was settling, and the colloids were separated as described in the foregoing and weighed. By plotting the hydrometer readings against the corresponding temperatures and drawing the curve, one obtains the correct value at 67°F. The difference between this

TABLE 3

Calculated average hydrometer readings for all soils and the corresponding computed corrections

CONCENTRATION PER LITER WATER	TEMPERATURE	HYDROMETER	CORRECTION PER DEGREE F.
gm.	°F.	°F.	
10	50	7.35	−0.08
	55	7.05	−0.08
	60	6.75	−0.10
	65	6.30	−0.12
	67	6.05	.....
	70	5.50	+0.13
	75	4.95	+0.14
	80	4.10	+0.15
	85	3.10	+0.16
50	50	27.45	−0.12
	55	26.95	−0.13
	60	26.35	−0.14
	65	25.65	−0.15
	67	25.35	.....
	70	24.75	+0.20
	75	23.85	+0.19
	80	22.75	+0.20
	85	21.35	+0.22
100	50	50.95	−0.17
	55	50.30	−0.19
	60	49.45	−0.21
	65	48.45	−0.22
	67	48.00	.....
	70	47.40	+0.20
	75	46.25	+0.22
	80	45.00	+0.23
	85	43.65	+0.24

value and the various hydrometer readings, divided by the difference between 67°F. and the observed temperature, gives the correction that has to be applied for each degree F. to obtain the correct value (table 2).

For hydrometer readings which were very near 67°F. i.e., within one degree above or below, the correction has not been computed, because in these cases the experimental error involved in both the hydrometer and temperature reading may cause too great a deviation in computing the correction.

The values given in table 2 were averaged for all soils and are given in table 3. These composite averages are also shown graphically in figure 3, where the relation of the density of water at different temperatures to the hydrometer reading at different temperatures is also given.

Several things become apparent from these data. As is seen, the form of curve obtained when the hydrometer readings are plotted against the cor-

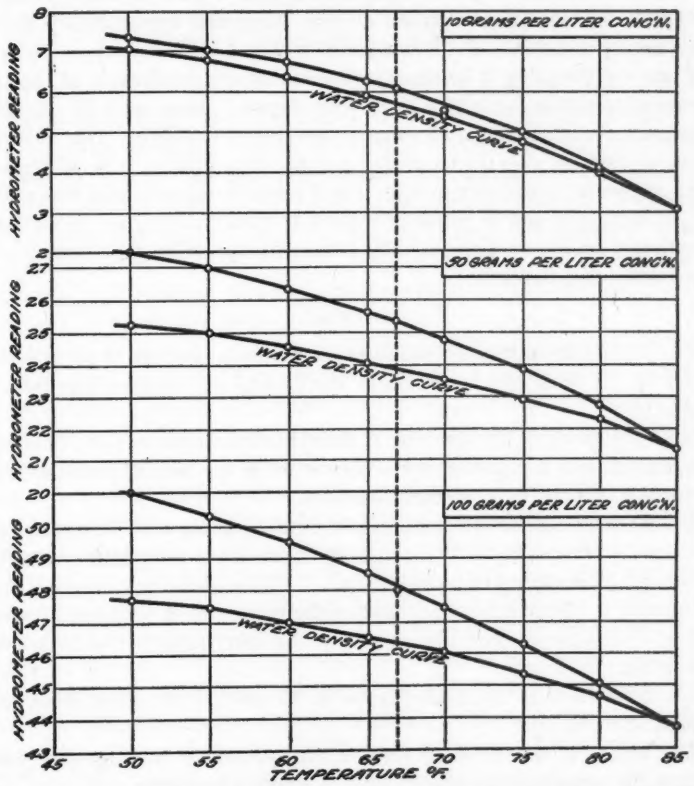

FIG. 3. CORRELATION BETWEEN TEMPERATURE AND HYDROMETER READING AT DIFFERENT CONCENTRATIONS AND THEIR RELATION TO THE DENSITY OF WATER AT DIFFERENT TEMPERATURES

Composite average of five soils

responding temperatures resembles closely that of the density of water when plotted against the temperatures. The slope of the hydrometer curve approaches that of the water-density curve at the low range of the hydrometer.

The corrections obtained for each degree F., as shown in the last column of table 3, indicate that: (*a*) at any one temperature the correction to be applied

varies with the hydrometer reading, i.e., it becomes smaller as the hydrometer reading decreases, as was shown in table 1; (*b*) at any one concentration the correction to be applied varies with the temperature, i.e., the correction increases as the temperature increases; (*c*) the correction for each degree F. below 67°F. is smaller than above 67°.

As is seen, the correction obtained for each degree F. at 85°F., as shown in the last column of table 3 (i.e., +0.16, +0.22, and +0.24 respectively) agrees well with the corrections obtained at that range and approximately at that temperature in the case of soil 5, as given in table 1.

From the foregoing it becomes apparent that the application of the temperature correction factor of 0.35 for each degree F. does not hold true at all hydrometer readings and at all temperatures, but that a sliding scale correction table is desirable with the method to increase its accuracy. The sliding scale table suggested tentatively in table 1 and figure 2 may not hold true exactly for all soil types and is probably subject to modifications, but nevertheless it indicates the nature of the rule which has to be applied in the construction of these tables of corrections.

SUMMARY

The rôle of concentration of soil suspension and temperature in the correction factor of the hydrometer method of mechanical soil analysis has been investigated.

Soil suspensions of the same soil at different concentrations but at the same temperature will not give identical results when the temperature correction factor of 0.35 is applied at all concentrations.

The same soil suspension which gave a certain reading at 67°F. will not give the same result at a higher or lower temperature if the temperature correction factor of 0.35 is applied unconditionally.

In order to obtain concordant results, the correction to be applied for each degree F. becomes considerably smaller at the lower range of the hydrometer than at the higher range.

The corrections to be applied below 67°F. are different from those to be applied above 67°F.

The nature of the curve obtained when hydrometer readings are plotted against the corresponding temperatures is graphically shown.

The sliding scale nature of a correction table to give concordant results at all concentrations is pointed out.

A sliding scale correction table is tentatively suggested, subject to modification.

INTERACTION BETWEEN AMMONIA AND SOILS, AS A NEW METHOD OF CHARACTERIZING SOIL COLLOIDS

AMAR NATH PURI

University Chemical Laboratory, Lahore, India

Received for publication May 1, 1930

In earlier publications (4, 5) the author has shown that the so-called adsorption of ammonia by soils is a chemical reaction representing the neutralization of acidoid by a base. Methods of estimating soil colloids based on ammonia absorption therefore cannot be employed for the purpose intended. It seemed, however, that this reaction could be used for finding the base neutralizing power of acidoids in different soils (6). The object of this investigation was to explore the possibilities of this reaction as a means of differentiating between soil colloids from various sources.

Interaction between bases and soil acidoids, is a slow process; and several days may be required for equilibrium to be reached. This is shown in a striking manner in table 1, which records the speed of reaction as measured by variations in the pH values of soil suspensions to which different amounts of NaOH and $Ba(OH)_2$ were added. The soil was first treated with 0.05 *N* HCl exaustively to bring it to the fully unsaturated condition (acidoid state).

It will be seen from table 1 that the speed of reaction depends on the strength of the base used; and equilibrium could be reached fairly quickly by using excess of alkali. It seems, therefore, that the best method of bringing the soil to the saturation or neutralization point would be to add excess of alkali and then remove the excess by leaching or precipitating as an insoluble salt. This point is recognized in the author's method of finding saturation capacity of soils for bases (6) where $Ba(OH)_2$ solution is added and excess removed by titration to phenolphthalein with 0.1 *N* H_2SO_4. The following method was used to bring about a similar state of affairs in the reaction with ammonia.

Ten grams of soil is stirred with about 50 cc. of water. Enough 0.2*N* HCl to break up all the carbonates is added gradually and the mixture set aside with frequent shaking till no more bubbles of CO_2 are formed. The soil is then transferred to a filter paper and washed with 0.05*N* HCl till the filtrate shows no reaction for Ca ions; this is followed by leaching with water till the soil is free from Cl ions. It is then washed with a little alcohol and air dried. The final washing with alcohol makes the soil loose and friable as well as easily detachable from the filter paper.

For washing purposes, a Büchner funnel is generally convenient; though a better method is to use filtering apparatus described by the writer elsewhere (3), with the modification that the cylinder portion need only be 2 or 3 inches long and provided with a lip.

After being air dried the soil is transferred to a flat dish. If a hardened filter paper, like

the Watman 50, is used the soil comes off quantitatively, with the help of a spatula and camel's hair brush. The dish containing the soil is kept over normal ammonia in a vacuum desiccator for two days. It is then transferred to another desiccator and kept over 90 per cent H_2SO_4 under vacuum for 48 hours. The ammonia retained by the soil is then determined by distillation with lime in the usual way.

DISCUSSION OF RESULTS

The results of ammonia reaction with 45 soils are recorded in table 2, which also gives the clay contents and pH values of the original and of the ammonia saturated soils.

It is seen that except in the case of soils free from carbonates (acid soils) or those containing Na_2CO_3 (alkali soils), the pH value of the original soil is approximately the same as after acid treatment and ammonia saturation. The reason is not hard to find. In almost all cases where the agreement is apparent, the soil contains excess of $CaCO_3$ and has reached a state of satura-

TABLE 1

Speed of reaction between soil and $Ba(OH)_2$ and NaOH

(10 gm. of soil kept with 100 cc. alkali solution)

M.EQ. OF ALKALI	pH AFTER							
	1 day	2 days	4 days	6 days	7 days	10 days	11 days	22 days
(NaOH)								
2	5.98	5.30	...	5.06	...	4.86	...	4.63
5	9.18	7.20	...	6.85	...	6.71	...	6.45
10	11.74	11.60	...	11.60	...	11.57	...	11.57
$Ba(OH)_2$								
2	4.64	4.23	4.00	...	3.83	...	3.75	3.65
5	7.85	7.00	6.49	...	6.00	...	5.90	5.74
10	11.00	10.80	10.66	...	10.65	...	10.60	10.57

tion with respect to Ca, in the sense that any excess of the Ca added to it will either be converted into carbonate or washed down if the soil is subjected to leaching. In other words it represents a limit beyond which any base, if present, will be easily hydrolyzed and removed by leaching.

The mechanism of ammonia reaction is similar: the soil is allowed to take up an excess and the easily hydrolyzable portion is removed under vacuum over H_2SO_4. Thus the soil come to equilibrium at a pH value which is not far different from that found under natural condition in the presence of excess $CaCO_3$. However in the case of soils having no $CaCO_3$, or containing alkali salts, the pH value in the natural state may be too low or too high as compared to that obtained after ammonia saturation.

It must be pointed out, however, that the difference between hydrolyzable and non-hydrolyzable bases is only of degree; and there is no reason to suppose that almost all the bases will not be removed, provided the leaching action is

TABLE 2

Interaction between ammonia and various soils

SOIL	CLAY (0.002 MM.)	NH_3 ABSORBED		NH_3 SATURATED SOIL	ORIGINAL SOIL
		Per 100 gm. soil	Per gm. clay		
	per cent	*m.eq.*	*m.eq.*	*pH*	*pH*
1	11.3	5.3	0.469	8.21	8.45
3	62.2	63.1	1.015	8.01	7.64
4	15.2	8.2	0.539	8.40	8.55
5	12.3	7.32	0.595	8.44	8.77
6	28.4	11.0	0.387	8.14	5.29
7	21.8	7.1	0.326	8.88	9.58
8	25.2	18.7	0.742	8.36	8.41
9	21.6	8.1	0.375	7.74	5.76
10	35.6	25.1	0.705	8.28	8.71
11	32.8	23.7	0.722	8.55	8.77
12	7.2	4.6	0.639	7.83	5.83
15	22.4	14.3	0.638	7.97	7.71
16	8.7	4.9	0.563	8.44	8.74
17	14.1	8.2	0.581	8.32	8.20
18	22.6	11.7	0.518	8.19	5.79
19	42.3	24.05	0.569	8.46	8.40
20	8.1	4.4	0.543	8.12	5.64
21	13.5	12.9	0.955	8.37	8.25
22	15.1	12.7	0.841	8.29	6.85
23	11.3	11.1	0.982	8.24	7.41
24	9.7	8.8	0.907	8.51	8.59
25	4.0	2.1	0.525	8.65	7.40
26	22.5	5.7	0.253	8.41	8.11
27	53.2	51.3	0.964	8.52	9.03
29	63.0	49.1	0.779	8.54	8.05
30	54.1	57.5	1.063	8.59	8.45
31	22.8	17.1	0.75	8.26	8.01
32	64.6	43.9	0.68	7.88	5.05
33	2.6	2.4	0.923	8.57	10.18
34	11.3	7.0	0.620	8.39	7.63
35	18.3	11.6	0.634	8.50	7.98
36	11.7	6.85	0.585	8.51	8.46
37	4.1	7.60	1.853	8.38	5.72
38	52.9	47.3	0.894	8.47	8.29
39	8.4	11.9	1.417	8.49	9.11
40	13.1	11.0	0.84	8.54	7.65
41	53.4	51.7	0.968	8.63	8.74
42	53.4	56.3	1.054	8.90	9.00
43	21.6	15.3	0.708	8.62	8.41
44	8.4	6.5	0.774	8.73	8.54
45	11.1	5.8	0.522	8.59	7.45
46	56.4	51.2	0.908	8.51	7.63
48	19.8	4.7	0.237	8.57	8.55
49	27.3	17.0	0.623	8.15	6.33
50	17.7	9.6	0.542	8.51	8.54

sufficiently prolonged. Another point worthy of note in this connection is that the removal of bases by simple leaching must depend entirely on the mutual reaction of the two bodies, i.e. only if the pH value of water is lower than that of the suspension of soil in the water, will bases be removed by leaching with that water.

It is interesting to note that the pH value at the saturation point for most soils is somewhere about 8.5, but it is appreciably lower in the case of all humus and latarite soils. It would thus appear that acidity in such soils is not only produced by their peculiar geographical condition of being situated in humid regions, but the nature of acidoids in them is such that if converted into saloids, they would tend to hydrolyze at a lower pH value and thus become acid much more readily than others.

Alkali soils, on the other hand, generally show quite the opposite effect: their hydrolysis point is situated at a higher pH value, so that such soils by the very nature of things, would resist the removal of bases by leaching, and tend to remain alkaline.

It is customary to denote the base exchange capacity of a soil by the amount of total cations (including H) removable by neutral salt reaction. Such a procedure is not logically sound, since the total exchangeable H cannot be removed by neutral salt reaction because it can never raise the pH value of the soil above 7. Bases removed by neutral salt reaction can only be spoken of as exchangeable bases, and should not be confused with base exchange capacity: just as moisture content of a soil is not the same as its moisture holding capacity. The one represents a dynamic state, the other a static condition.

It is evident that ammonia reaction affords a simple method of determining base exchange capacity of soils, and, as such, could be used as a "single value" for characterizing them in any system of genetic classification. It has been generally recognized that colloids separated from different soils are not quite alike; though they show a similarity of behavior on the whole. It is hoped that the method outlined will prove of some value in bringing out such differences.

Although no attempt can be made at this stage to show that similarly constituted soils can be grouped together on the basis of base exchange capacity, it might be stated that, on the whole, dark colored soils show a higher value than red, lateritic, or humus soils. A somewhat similar conclusion has already been arrived at by Mattson from his extensive researches on the colloidal behavior of soils with varying silica sesquioxide ratios (1).

Reference has already been made to the fact that most of the soils in nature, in the presence of $CaCO_3$, tend to acquire a pH value of 8.5, which may be approximately taken as the limit beyond which bases, if combined, would be easily hydrolyzed. It is therefore conceivable that this stage of reaction might mark the maxima or minima of certain soil properties. The author's conclusion, arrived at elsewhere (2), that to produce maximum dispersion of

soil for mechanical analysis the suspension must be made alkaline to phenolphthalein, is thus rendered comprehensible.

SUMMARY

Soils react with bases very slowly, and equilibrium condition can be reached quickly only by adding excess of alkali and removing the excess by leaching or by precipitation as some insoluble salt.

A soil made completely unsaturated can combine with an amount of ammonia which may be considered equivalent to its base exchange capacity.

The pH value of soil containing excess $CaCO_3$, after dilute acid treatment and ammonia saturation is approximately the same as that of the soil in the natural condition.

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DETERMINATION OF THE PERCENTAGE BASE SATURATION OF SOILS AND ITS VALUE IN DIFFERENT SOILS AT DEFINITE pH VALUES[1]

W. H. PIERRE AND G. D. SCARSETH[2]

Alabama Agricultural Experiment Station

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Hissink (12) in his early work on base exchange in relation to soil reaction emphasized the importance and necessity of considering the degree to which the soil exchange complex is saturated with bases. He introduced into soil science the term "degree of saturation of soils." Numerically expressed, this is known as the percentage base saturation. Satisfactory procedures for the determination of base saturation have been slow in development, however, and the work on base exchange has dealt more with considerations of the exchangeable bases than of the exchangeable bases in relation to the total exchange capacity of soils, i.e. percentage base saturation. As far as soil reaction is concerned, the relation between the exchangeable bases or the exchangeable hydrogen and the total exchange capacity is far more important than is the knowledge of any one of these values considered alone.

Since soil acidity is due to the presence of exchangeable hydrogen, it might be supposed that a close correlation would exist between the hydrogen-ion concentration of soils and their percentage base saturation. Green (9) reports that he obtained a good correlation between the hydrogen-ion concentration of soils and their percentage base saturation. On the other hand, Joffe and McLean (13) state as a result of their work that the hydrogen-ion concentration of the water extract of soils adds little to the knowledge of the state of saturation. Very little data are presented by these investigators, and the fragmentary data obtained by other investigators aid little in clarifying this question. The recent development of more satisfactory methods, however, makes possible a more thorough study of the problem.

Not only is it desirable to study the relation between hydrogen concentration and percentage base saturation, but it is also of fundamental importance to study the relation between percentage base saturation of soils and plant growth. This paper will deal with the first of these problems. The latter problem has been given considerable study by one of the writers, and the results are presented in an accompanying paper (25).

[1] Published with the approval of the director of the Alabama Agricultural Experiment Station.

[2] Associate soil chemist and assistant soil chemist, respectively.

DETERMINING THE PERCENTAGE BASE SATURATION OF SOILS

Three values—those for the exchangeable hydrogen, the total exchangeable bases, and the total exchange capacity—may be concerned in determining the percentage base saturation of soils. Obviously, if any two of these values are known the third can be calculated as can also the value for percentage base saturation. Thus any one of the following procedures may be used for the determination of percentage base saturation of soils:

a. Determinations of total exchangeable bases and of exchangeable hydrogen.
b. Determination of total exchangeable bases and of total exchange capacity.
c. Determinations of exchangeable hydrogen and total exchange capacity.

Each of these procedures has been used by one or more investigators. No attempt will be made here to describe the various methods that have been used in studies of the base saturation of soils. Some interesting comparisons of methods have been made by Gericke (8), Harada (10), and Turner (29). It is obvious that the accuracy of the percentage base saturation data obtained will depend on the accuracy of the separate determinations used in obtaining the values.

Because of the difficulty, during the early investigations on base exchange, of determining the total exchange capacity or the saturation capacity of soils, procedure (a) was the one employed by Hissink (12) and Gedroiz (7) in their pioneer investigations. Later work by Kelly and Brown (15), Parker (19), and others showed, however, that the methods for determinations of exchangeable hydrogen as used by these early investigators were inaccurate. Moreover, it was found that determinations of the total exchangeable bases may be subject to a considerable error because of the solution of non-exchangeable bases by the neutral salt solution used for leaching (17, 23). No very satisfactory method was at first available, therefore, for determining the degree of saturation of soils.

More recent work has resulted in a better understanding of the base exchange reaction of soils, and with this better understanding have come better methods. Among the best known of these have been the method developed by Kelly and Brown (18) for determining the total exchange capacity and the methods of Parker (19) for determining the exchangeable hydrogen. Other methods for determining one or the other of the values required in calculating the degree of saturation of a soil have been proposed by various investigators (3, 10, 26, 29). Schollenberger's ammonium acetate method (26) of determining both exchangeable hydrogen and total exchange capacity is of special interest because of its relative simplicity. Instead of leaching the soil with barium acetate, as in Parker's method, he leached the soil with ammonium acetate and titrated the leachings in order to determine the exchangeable hydrogen content. Since the exchange complex was believed to be saturated with ammonium by this treatment, he determined the amount of ammonia absorbed as a measure of the total exchange capacity. In the experience of the writers, considerable diffi-

culty was found in getting good duplication in determining exchangeable hydrogen by this method, especially on sandy soils, where the amounts determined were small. For this reason Parker's method of determining exchangeable hydrogen seemed preferable. Schollenberger's method, however, makes possible the determination of exchangeable hydrogen and of total exchange capacity on the same soil sample and with few operations. The writers, therefore, studied the possibility of adapting Parker's barium acetate method of determining exchangeable hydrogen for the determination of the total exchange capacity somewhat on Schollenberger's principle. Such a method, if proved satisfactory, would be more simple and rapid than the method of Kelly and Brown (18), especially if the exchangeable hydrogen of the soil was also to be determined.

The method consisted of leaching 10 gm. of soil with 250 cc. of *N* barium acetate of pH 7.0 and titrating the leachate for exchangeable hydrogen. The soil was then leached with 250 cc. of neutral *N* NH_4Cl in order to replace the barium by ammonium, and the excess NH_4Cl was removed by washing with 95 per cent ethyl alcohol until chlorides no longer appeared in the leachate. The absorbed ammonia was then determined by transferring the soil into a kjeldahl flask, distilling with magnesium oxide, and titrating the distillate with 0.1 *N* H_2SO_4. In the following discussion this method will be referred to as the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method of determining total exchange capacity.

This method was compared with the $Ba(OH)_2 - NH_4Cl$ method of Kelly and Brown as slightly modified by Parker. Some of the results obtained have already been given by Parker (22). It was found that the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method almost invariably gave lower values than did the $Ba(OH)_2 - NH_4Cl$ method. The differences in the values obtained by the two methods, although not large numerically, were significant from a percentage standpoint and caused considerable difference in the values for percentage base saturation, especially with sandy soils of low total exchange capacities. This will be noted from a study of the data given in columns two and three of table 1 and also in columns three and four of table 2. In table 1 are also given the percentage base saturation values for the 15 Coastal Plain soils, calculated on the basis of these two methods of determining total exchange capacity. These soils had been limed six months previously with precipitated calcium carbonate at the rate of one ton to the acre in greenhouse pots. Since determinations of exchangeable hydrogen were made on both the limed and unlimed soil, this made possible calculations of the percentage base saturation at two uniform pH values. The results are given in the last four columns of table 1. It will be noted that significant differences were obtained in the percentage base saturation values, depending on which method was used in determining the total exchange capacity. This was especially true at the lower pH value.

It is evident that either the $Ba(OH)_2 - NH_4Cl$ method gives high values, the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method gives low values, or that there are discrepancies in both methods of determining total exchange capacity. Parker (22) has

suggested the former possibility on the basis that very drastic treatments of the soil with $Ba(OH)_2$ may further increase its total exchange capacity. On the other hand, Kelly and Brown (18) used a preliminary treatment with an alkaline solution because they believe that the affinity of the hydrogen ions for the exchange complex, particularly the organic exchange complex, is so great that complete reversibility is facilitated by the use of an alkaline solution.

In order to make a further study of these two methods of determining total exchange capacity, 10 of the soils used in this investigation were limed at two different rates, one calculated to bring them to neutrality and the other to above neutrality. After one year, when the calcium carbonate had come to equilibrium with the soil, determinations of total exchange capacity were made by the two methods on the treated and on the limed samples. The results are given in table 2. It was found that the total exchange capacity values as determined by the $Ba(OH)_2 - NH_4Cl$ method were practically the same for the limed and the unlimed samples of each soil. On the other hand, the values obtained by the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method were greater with the limed soils than with the untreated soils. In the last two columns of the table are given the percentage differences in the total exchange capacity values obtained by the two methods. It will be noted that whereas the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method gave values considerably lower than did the $Ba(OH)_2 - NH_4Cl$ method on the unlimed soils, it gave values for the limed soil which were very similar to those obtained by the latter method.

These results can be explained on two different hypotheses. In the first place, it may mean that liming a soil, like treating it with an alkaline solution as in the $Ba(OH)_2 - NH_4Cl$ method, causes an increase in the total exchange capacity of the soil. On the other hand, the results might be explained on the basis of more complete replacement of hydrogen by the $Ba(OH)_2 - NH_4Cl$ than by the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method. This would mean that the exchangeable hydrogen values obtained by the barium acetate method are low. The true exchangeable hydrogen values would then be represented by the sum of the hydrogen obtained by the barium acetate method and that which is further replaced by an alkaline solution as measured by the increase in total exchange capacity obtained by the $Ba(OH)_2 - NH_4Cl$ over that by the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method. It is interesting to note in table 3 that the percentage base saturation values of the soils at pH 4.80 based on these two hypotheses are very similar. These values are quite different, however, from those obtained by using the results from the $Ba(OH)_2 - NH_4Cl$ method for total exchange capacity and the barium acetate method for exchangeable hydrogen. The writers believe that the first hypothesis is tenable and that the $Ba(C_2H_3O_2)_2 - NH_4Cl$ gives a better value for saturation capacity than does the $Ba(OH)_2 - NH_4Cl$ method. In the first place, the studies of Burgess (5), Magistad (19), and others lend considerable evidence to the belief that the total exchange capacity of a soil can be increased by treating it with an alkaline solution. Secondly, Parker (22) has shown that the barium acetate method

gives very similar values for exchangeable hydrogen as do two other methods based on different principles. Moreover, he found that the soils were approximately neutral in reaction after being leached with neutral barium acetate. The conception that the barium acetate method gives true values for exchangeable hydrogen is also believed to be in better harmony with the meaning of a saturated soil. Although there is some difference in opinion as to the meaning of the saturation or total exchange capacity of soils the writers are in agreement with the definition given by Bradfield (3) that saturation capacity refers to the limit to which a soil can be saturated with bases by means of neutral salts.

It may be tentatively assumed, therefore, that the higher total exchange capacity values obtained by the $Ba(OH)_2 - NH_4Cl$ method than by the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method and the higher values obtained on limed than on unlimed soil by the latter method, are caused by a so-called "build-up" or increase in the total exchange capacity of the soil. For this reason the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method of determining total exchange capacity and the barium acetate method for exchangeable hydrogen were used in this investigation in calculating the percentage base saturation of the soils at the different pH values.

THE PERCENTAGE BASE SATURATION OF SOILS AT DEFINITE pH VALUES

In table 1, to which reference has already been made, are given the percentage base saturation values of 15 sandy Coastal Plain soils. Since limed and unlimed samples of these soils were available, it was possible to calculate these values at two different pH values. The calculated values for percentage base saturation at pH 5.55 and pH 6.0 are given in the ninth and in the last columns of this table. It will be seen that at pH 5.55 the percentage base saturation values ranged from 27.0 to 48.9, and at pH 6.0 beween 43.1 and 62.4. These data show that the soils of similar origin and degree of weathering may differ considerably in their degree of saturation at like pH values.

Soils formed under different climatic conditions and of various degrees of weathering had been studied by one of the writers in some accompanying studies of the relation between soil acidity and plant growth (24) and also in studies of the buffer capacity of soils. The soils investigated had each been brought to different degrees of acidity in pots in the greenhouse, by treatment with precipitated calcium carbonate or with acid. The treatments of the first 13 soils of table 4 are fully described in an accompanying paper (24). The last two soils of table 3 were originally very acid and therefore had received only lime treatments. In all cases the lime was added in the form of precipitated calcium carbonate. Both the lime and the acid were allowed to react with the soil in a moist condition for nine months before the studies of percentage base saturation were made. The hydrogen-ion concentration of the soils was determined by the "dialysis-colorimetric method" (25).

In table 4 are given the values for the percentage base saturation of the soils at five definite pH values. If, for example, the percentage base saturation

values at pH 4.80 are considered, it will be noted that there is a wide variation between soils in this regard. Whereas soils 674 and 675 were less than 10 per cent saturated with bases at pH 4.80, soils 670, 673, 686, and 641 were over 50 per cent saturated at this same pH value.

Similar differences in the percentage base saturation values of the various soils are obtained at the other pH values. As the soils approach neutrality,

TABLE 1

The percentage base saturation of soils based on two different methods of determining total exchange capacity

SOIL TYPE	TOTAL EXCHANGE CAPACITY		H-ION CONCENTRATION AND EXCHANGEABLE HYDROGEN OF SOILS				PERCENTAGE BASE SATURATION BASED ON DIFFERENT METHODS AT pH VALUES OF			
			Unlimed soil		Limed soil		pH 5.55		pH 6.0	
	By the $Ba(OH)_2$ – NH_4Cl method	By the $Ba(C_2H_3O_2)_2$ – NH_4Cl method	pH	E. hydrogen	pH	E. hydrogen	$Ba(OH)_2$ – NH_4Cl method	$Ba(C_2H_3O_2)_2$ – NH_4Cl method	$Ba(OH)_2$ – NH_4Cl method	$Ba(C_2H_3O_2)_2$ – NH_4Cl method
	mgm. eq.	*mgm. eq.*		*mgm. eq.*		*mgm. eq.*	*per cent*	*per cent*	*per cent*	*per cent*
Norfolk sandy loam	4.24	3.54	5.45	2.40	6.00	1.33	48.0	37.7	68.6	62.4
Greenville loam	7.43	6.90	5.55	3.53	6.25	2.13	52.5	48.9	64.7	61.9
Orangeburg very fine sandy loam	7.25	5.60	5.55	3.40	6.15	2.20	53.0	39.3	65.5	55.3
Norfolk fine sandy loam	8.29	6.63	5.55	4.40	6.00	3.47	47.0	33.5	58.3	47.8
Orangeburg sandy loam	5.44	4.03	5.55	2.67	6.20	1.67	51.0	33.8	64.0	51.1
Susquehanna very fine sand	3.84	2.90	5.55	2.07	6.25	1.00	46.1	28.6	64.3	52.5
Norfolk sandy loam	4.05	3.02	5.30	2.47	6.25	1.47	45.3	27.0	57.1	43.1
Norfolk fine sand	3.19	2.50	5.05	2.20	6.20	1.20	44.4	28.9	57.0	45.1
Norfolk loam	5.19	3.88	4.95	2.94	5.65	2.13	56.7	42.1	....	....
Greenville sand	1.62	1.71	5.45	1.13	6.70	0.20	35.0	38.0	56.0	58.1
Greenville pebbly loam	2.85	2.70	5.40	1.60	6.60	0.73	47.6	44.5	59.3	57.0
Norfolk sandy loam	3.71	3.00	5.55	1.87	6.35	0.87	49.6	37.7	65.0	56.8
Norfolk sandy loam	2.85	2.52	5.40	1.60	6.30	0.80	48.4	41.8	62.8	57.9
Greenville sand	1.71	1.46	5.20	1.13	6.80	0.33	44.0	34.2	57.5	50.2
Orangeburg fine sand	1.86	1.43	5.40	0.87	6.85	0.20	56.0	42.7	68.0	58.5

the differences in the percentage base saturation values between the various soils become less. This should be expected if soils are completely saturated with bases at pH 7.0. It will also be noted that as the soils approach neutrality they approach complete saturation. These results are typical of results obtained on other soils. With most soils it has been found that complete saturation, as determined by the methods used, is reached at pH 7.0, which fact

TABLE 2

The total exchange capacity of untreated and of limed soils as determined by the Ba $(OH)_2$—NH_4Cl and the $Ba(C_2H_3O_2)_2$-NH_4Cl methods

SOIL NUMBER	UNTREATED SOIL			SOIL LIMED TO ABOUT NEUTRALITY		SOIL LIMED TO ABOVE NEUTRALITY			PERCENTAGE DIFFERENCE IN TOTAL EXCHANGE CAPACITY BY THE TWO METHODS	
	pH value	Total exchange capacity		pH value	Total exchange capacity	pH value	Total exchange capacity		Untreated soil	Soil limed to above neutrality
		$Ba(OH)_2$ — NH_4Cl method	$Ba(C_2H_3O_2)_2$ — NH_4Cl method		$Ba(C_2H_3O_2)_2$ — NH_4Cl method		$Ba(OH)_2$ — NH_4Cl method	$Ba(C_2H_3O_2)_2$ — NH_4Cl method		
		mgm. eq.	*mgm. eq.*		*mgm. eq.*		*mgm. eq.*	*mgm. eq.*	*per cent*	*per cent*
670	5.20	29.37	26.33	6.80	27.43	7.43	29.37	28.00	10.3	4.7
671	4.50	8.88	7.76	6.68	8.40	7.40	9.09	8.78	12.6	3.4
672	5.80	7.29	5.50	6.78	6.25	7.43	7.24	6.57	24.6	9.2
673	6.43	9.47	9.41	7.22	9.40	7.45	9.43	9.12	0.6	3.2
674	6.00	5.57	4.85	6.80	5.18	7.45	5.60	5.28	12.9	5.7
675	5.75	2.35	1.83	7.00	2.05	7.52	2.29	2.25	22.1	1.7
676	5.60	14.54	13.28	6.80	13.21	7.38	14.80	13.57	8.7	8.3
678	5.53	4.35	4.00	7.38	.....	7.43	4.64	4.28	8.0	7.8
679	5.80	10.68	9.79	6.93	10.43	7.38	10.71	10.65	8.3	0.6
680	5.38	8.26	6.97	7.00	7.65	7.45	8.19	7.75	15.6	5.4
Average...	5.60	10.08	8.97	6.94	.....	7.43	10.13	9.63	12.3	4.4

TABLE 3

The percentage base saturation of soils at pH 4.80 as determined by different methods

SOIL NUMBER	METHOD 1	METHOD 2	METHOD 3
670	57	52	62
671	32	28	41
672	10	8	32
673	77	76	77
674	6	5	19
675	9	7	28
676	32	29	37
678	20	18	28
679	43	40	45
680	18	15	31

Method 1 is based on the total exchange capacity determined by the $Ba(C_2H_3O_2)_2$—NH_4Cl method and on the exchangeable hydrogen determined by the barium acetate method.

Method 2 is based on the total exchange capacity determined by the $Ba(OH)_2$ — NH_4Cl method, and the exchangeable hydrogen obtained by the barium acetate method plus the hydrogen measured by the difference between the values for total exchange capacity obtained by the $Ba(OH)_2$ — NH_4Cl and the $Ba(C_2H_3O_2)_2$ — NH_4Cl methods.

Method 3 is based on the total exchange capacity determined by the $Ba(OH)_2$ — NH_4Cl method and the exchangeable hydrogen determined by the barium acetate method.

indicates that the exchange complex saturated with calcium as the principle exchange cation does not undergo much hydrolysis.

In general, the soils from the Coastal Plain and Piedmont Plateau Provinces are lower in percentage base saturation than the other soils which are younger and less highly weathered. Three apparent exceptions, however, were obtained. These were the two Susquehanna soils and the Octibbeha clay. All these soils had a higher degree of saturation at given pH values than the other soils of the Coastal Plain, and two of these had just as high values as most of the other soils studied. It is significant, however, that all three of these soils are from

TABLE 4

The percentage base saturation of soils of different origins at like pH values

SOIL NUMBER	SOIL TYPE	LOCATION OF SOIL	TOTAL EXCHANGE CAPACITY	PERCENTAGE BASE SATURATION AT pH VALUES OF				
				4.80	5.00	5.50	6.00	6.50
			mgm. eq.					
670	Grundy silt loam	Ill.	26.33	57	60	69	80	91
671	Cory silt loam	Ill.	7.76	32	38	56	74	88
672	Cecil sandy loam	S. C.	5.50	10	20	36	53	69
673	Delta light silt loam	Miss.	9.41	77	80	86	92	94
674	Cecil clay loam	Ala.	4.85	6	23	41	58	74
675	Norfolk sandy loam	Ala.	1.83	9	16	32	44	60
676	Colby silt loam	Wis.	13.28	32	37	54	66	78
678	Cecil clay	Ala.	4.00	20	39	52	68	85
679	Miami silt loam	Wis.	9.79	43	50	63	72	82
680	Greenville fine sandy loam	Ala.	6.97	18	21	34	54	71
683	Susquehanna fine sandy loam	Ala.	4.05	28	41	50	70	....
684	Norfolk sandy loam	Ala.	3.00	20	31	40	59	....
685	Greenville sandy loam	Ala.	2.33	....	43	59	70	84
686	Oktibbeha clay	Ala.	19.36	52	66	78	82	86
641	Susquehanna clay	Ala.	34.25	55	70	79	85	94
Average of all soils.....			10.18	33	43	55	68	81

the Coastal Plain Black Belt, and that they are less highly weathered than the other soils of the Coastal Plain or Piedmont Plateau provinces.

The values for percentage bases saturation at pH 4.80 for soils 672, 674, and 675 seem low. They are what might be expected, however, when it is considered that the pH values of the desaturated colloids from these soils were found by Baver and Scarseth (2) to be between 4.40 and 4.75, whereas those of the colloids from the soils giving higher degrees of saturation were pH 4.0 or less. On the other hand, the base saturation values for some of the other soils at low pH values seem high. It is difficult to understand, for example, how soil 673 can be 77 per cent saturated with bases at pH 4.80. Some evidence for the belief that this value may be high has been obtained by Baver and Scarseth

(2) who determined the percentage base saturation from the titration curve of the electrodialyzed colloid from this soil. They obtained by this method considerably lower degrees of saturation than are here reported. When the exchangeable bases in this soil at pH 4.75, determined by electrodialysis, were used for the calculation of percentage base saturation, however, very similar values were obtained to those reported in the present study. As a further check on this soil, the exchangeable bases of the treated soil having a pH value of 4.75 and of the soil having a pH value of 5.80 were determined by Kappen's method (15). The value obtained at pH 4.75 was 8.80 mgm. equivalents and at pH 5.80, 10.50 mgm. equivalents. The exchangeable hydrogen values for these two samples were 2.80 and 0.90 mgm. equivalents, respectively. It appears that the values for exchangeable bases are higher than those obtained by difference in the present study. This is probably due to a solubility of non-exchangeable bases by the 0.1 *N* acid used in Kappen's method. It is significant, however, that the percentage base saturation values based on Kappen's method for exchangeable bases and those based on the barium acetate method for exchangeable hydrogen agree closely. A further study of this soil is being made, but it appears from the evidence at hand that although the values obtained may be slightly high this soil has a high percentage base saturation at low pH values. Possible explanations for the difference in the percentage base saturation of different soils at like pH values will be considered in the next section.

It is interesting to compare briefly the data on percentage base saturation obtained in this study with some of those obtained by other investigators who have used different methods. Mention will not be made here of data based on Hissink's original method, for Harada (10), Turner (29), and others have shown that his method gives low values for percentage base saturation.

Page and Williams (20) obtained base saturation values of 7.9 per cent at pH 4.04 and 29.0 per cent at pH 4.48 for soils from the Rothamsted Grass Plats.

Joffe and McLean (14) reported the percentage base saturation value for six soils and their subsoils of the Chenango series. Three of the soils had pH values of 5.0, and the percentage base saturation values were 43.70, 49.30, and 36.92. The subsoils showed a higher degree of saturation than the surface soils of similar pH values.

Conrey and Schollenberger (6), using the method previously referred to, determined the percentage base saturation of various horizons of a Clermont silt loam. They also obtained some evidence that the deeper subsoil is more highly saturated than the surface soil at the same hydrogen ion concentration. The surface soil at pH 5.92 was 48 per cent saturated, whereas the soil sample at 36 to 48 inches at the same pH value was found to be 81 per cent saturated with bases.

Harada (10), working with Japanese soils, reported percentage base saturation values for 16 soils whose pH values ranged from 4.48 to 6.43. The percentage base saturation values ranged from 13.6 with a soil of pH 5.94 to 77.2

with a soil of pH 6.0. Although he states that the degree of saturation can be correlated with the pH values, it is evident from his results that soils of the same pH values may have very different degrees of saturation.

Although close agreement cannot be expected between the results obtained by these various investigators and those reported in this study because of differences in soils and in the methods used, nevertheless, the data tend to substantiate the fact that soils of the same pH values may be very different in regard to percentage base saturation.

The differences obtained in the percentage base saturation of various soils at like pH values are of considerable practical as well as theoretical importance. These results show very definitely that the hydrogen ion concentration of soils does not truly represent the condition of soils with respect to acidity. In an accompanying paper (24) it is shown that within certain limits the growth of acid-sensitive crops on acid soils is much more closely correlated with the percentage base saturation than it is with the hydrogen-ion concentration of the soils. Likewise, it may be true that the biological activities of soils are more dependent on the degree of saturation of the soils than on their hydrogen-ion concentration.

FACTORS AFFECTING THE PERCENTAGE BASE SATURATION OF SOILS AT DEFINITE pH VALUES

In explaining the fact that soils differ greatly in their percentage base saturation at given pH values, the following factors should be considered:

Presence of soluble acids
Nature of the bases in the exchange complex
Nature of the exchange complex as might be revealed by
  a. Organic matter content of the soil
  b. Silica-sesquioxide ratio of soil colloid
  c. Total exchange capacity of soil colloid
Strength or avidity of the soil acids.

It might be expected that the presence of soluble acids in a soil would cause a relatively high degree of saturation at given pH values. Although most of the soils used in this study received acid treatments they were thoroughly leached to remove the soluble acids, and cropped to corn before they were studied. Some evidence for the effect of soluble acids was noted, however, with some other sandy soils which had been heavily fertilized with ammonium sulfate in the greenhouse and not subjected to leaching. Under ordinary conditions, however, the presence of soluble acids is not an important factor and cannot explain the major differences between soils obtained in this study.

A study was made of the nature of the bases in the exchange complex of some of the soil colloids. Since soils containing in the exchange complex monovalent bases, such as sodium, hydrolyze to a greater extent than do those containing divalent bases, such as calcium, it might be expected that the relative proportion of the various bases in the exchange complex would have some influence

on the percentage base saturation of soils at like pH values. It was found that calcium and magnesium constituted the greater portion of the exchangeable bases of all the colloids, and no correlation could be established between the proportion of monovalent to divalent bases and the degree of saturation at like pH values. For example, soil colloids 673 and 683 had higher proportions of monovalent to divalent bases than most of the other colloids and yet their percentage base saturation at given pH values is high. The data obtained, however, are not conclusive, for the colloids were of different degrees of saturation. It is very probable that if a soil contains a large proportion of exchangeable sodium, it would, because of hydrolysis of the exchange complex, show a lower degree of base saturation at the various pH values than a similar soil containing a greater proportion of exchangeable calcium.

The fact that soils vary considerably in percentage base saturation at like pH values is not surprising, however, if it is considered that the nature of the exchange complex of various soils may be quite different, and that the acid in the exchange complex of one soil may differ from that in the exchange complex of another soil. It seemed of interest, therefore, to study briefly some soil properties which might be correlated with the nature of the soil acids present and consequently with the percentage base saturation of soils at like pH values.

Since the organic matter of soils has base exchange properties, it might be argued that the percentage base saturation of the organic exchange complex is different from that of the inorganic exchange complex at like pH values. Soils with a high content of organic matter might, therefore, be expected to give different percentage base saturation values at a certain hydrogen-ion concentration than soils which have a very low content of organic matter. A study of the organic matter content of some of the soils investigated, however, failed to show any relationship between the percentage of organic matter and the percentage base saturation of the soils at like pH values. This will be noted by referring to table 1 of the accompanying paper (24). It is recognized, however, that a determination of total organic matter probably gives little indication of the organic matter that is active in base exchange or of the nature of the organic acids present.

Parker and Pate (23) and Anderson and Mattson (1) have shown that the silica-sesquioxide ratio of soil colloids bears a relation to their base exchange properties. That being true, it was thought that some correlation might be found between the silica-sesquioxide ratio of the soil colloids and the percentage base saturation of the soils at like pH values. Eleven soil colloids, which had been extracted by means of the supercentrifuge, were analyzed and their silica-sesquioxide ratios calculated. The results obtained are presented in table 5. A study of the ratios obtained and of the percentage base saturation values at like hydrogen-ion concentrations shows that in general soils of a high ratio seem to have higher degrees of saturation than soils with colloids of low silica-sesquioxide ratio. For example, the five soils which have the highest silica-sesquioxide ratio are also the five soils which have the highest degree of satura-

tion at pH 4.80. It is also significant that these five soils are less highly weathered than the other six soils showing a lower degree of saturation and a lower silica-sesquioxide ratio.

TABLE 5

The silica-sesquioxide ratio and total exchange capacity of extracted soil colloids as related to the percentage base saturation of the soils at pH 4.80

SOIL NUMBER	SOIL TYPE	TOTAL SiO_2	TOTAL Al_2O_3	TOTAL Fe_2O_3	$\frac{\text{MOLS } SiO_2}{\text{MOLS } R_2O_3}$	TOTAL EXCHANGE CAPACITY	BASE SATURATION OF SOIL AT pH 4.80
		per cent	*per cent*	*per cent*		*mgm. eq.*	*per cent*
670	Grundy silt loam	41.32	17.85	8.75	2.99	..	57
671	Cory silt loam	49.31	21.54	7.23	3.20	22	32
672	Cecil sandy loam	39.79	32.25	8.99	1.78	14	10
673	Delta light silt loam	49.32	25.67	9.16	2.66	32	77
674	Cecil clay loam	34.35	29.13	12.55	1.57	13	6
675	Norfolk sandy loam	41.01	32.53	5.75	1.92	22	9
676	Colby silt loam	47.43	20.96	6.80	3.18	32	32
678	Cecil clay	36.00	33.45	12.38	1.48	15	20
679	Miami silt loam	52.90	18.02	7.15	3.97	30	43
680	Greenville fine sandy loam	39.83	33.95	8.86	1.62	20	18
683	Susquehanna fine sandy loam	39.55	26.69	7.44	2.01	33	28

TABLE 6

The strength or avidity of soil acids as related to the percentage base saturation of soils at pH 4.80

SOIL NUMBER	H-ION CONCENTRATION	EXCHANGEABLE HYDROGEN CONTENT	AVIDITY* OF EXCHANGEABLE HYDROGEN	PERCENTAGE BASE SATURATION AT pH 4.80
	pH	*mgm. eq.*		
670	4.80	11.15	22.4	57
671	4.85	5.25	18.0	32
672	4.80	4.95	12.8	10
673	4.80	2.20	20.0	77
674	4.75	4.75	13.8	6
675	4.75	1.70	16.5	9
676	4.80	9.10	20.5	32
679	4.93	5.15	18.0	43
680	4.80	5.70	14.4	18
683	4.85	2.57	17.0	28
684	4.80	2.47	14.6	20

* Avidity is the actual percentage of total H replaced by the base K when the amount of $KC_2H_3O_2$ used is chemically equivalent to the amount of exchangeable H.

The total exchange capacity of the soil colloids are also given in table 5, and it will be noted that there is also a general relationship between these values and the percentage base saturation of the soils at pH 4.80. A very definite

correlation, however, between the silica-sesquioxide ratio or the total exchange capacity of the soil colloids and the percentage base saturation of the soils at given pH values is not obtained and is probably not to be expected, for the former values can be considered only partially indicative of the chemical nature of the exchange complex.

Various investigators have shown that the acids found in different soils or even in the same soil may be quite different. Truog (28) in his early work on soil acidity found that the acids in different soils vary in strength or avidity, and he proposed a method for determining the strength or avidity of soil acids based on the competition of the soil acids and of acetic acid for the base, potassium. Later, Tidmore and Parker (27) and Parker (22), using Truog's principle, also obtained marked differences in the strength of acids found in different soils. Parker also obtained evidence that the acids in the exchangeable condition in the same soil may be quite different. These results might be taken to indicate that soil acids differ in their degree of dissociation. If that is true, the hydrogen-ion concentration of soils can be looked upon as dependent on the percentage base saturation and on the degree of dissociation of the soil acids. Soils whose acids have a high degree of dissociation would, therefore, have at any definite pH value a higher degree of saturation than soils whose acids have a lower avidity or are less dissociated.

Studies were made of the avidity of the acids of the soils used in this study. For this purpose the soil samples at a pH of about 4.80 were used. The procedure used was that described by Parker (21) except that the soil suspensions treated with potassium acetate were allowed to stand over night before filtering. The data obtained are given in table 6. The results show very definitely that the avidity of the soil acids from the different soils varies considerably. Since these soils were at about the same pH values, it is evident that the hydrogen-ion concentration of soils does not give an entirely accurate picture of the strength of soil acids. The avidity of the exchangeable hydrogen or rather of the acids in the exchange complex varies from 13.8 with soil 674 to 22.4 with soil 670. The data show that the soils of the Piedmont Plateau and Coastal Plains which have undergone the greatest degree of weathering have acids of lower strength or avidity than do the other less weathered soils. Moreover, it is significant that the soils with acids of high avidity have a higher degree of saturation at pH 4.80 than do soils with acids of low avidity. This shows that the difference between the percentage base saturation of different soils at like pH values can to some extent be explained by the fact that the acids of different soils at like pH values may differ considerably in strength or avidity. Further evidence for this conclusion is obtained from the data given by Tidmore and Parker (27, p. 333) in which they found that the Susquehanna clay subsoil had relatively much stronger acids as measured by Truog's avidity method and by the sugar invesion test than by the determination of hydrogen-ion concentration. It will be remembered that the percentage base saturation of the Susquehanna clay used in this study, which was obtained from the same area

as was the one they used, gave a high degree of saturation at given pH values. The recent results obtained by Baver and Scarseth (2) are also very significant. They found that the relative strength of the acids found in some of the colloids extracted from these soils, as measured by multiplying the amount of acids present in the desaturated soil colloid by the dissociation constant of the acids, were very similar to the values for the relative strength of the acids found in this study.

SUMMARY

A study was made of the determination of the percentage base saturation of soils when based on the $Ba(OH)_2 - NH_4Cl$ and the $Ba(C_2H_2O_2)_2 - NH_4Cl$ methods of determining total exchange capacity. It was found that the $Ba(OH)_2 - NH_4Cl$ method gave values for total exchange capacity which averaged 17.9 per cent higher with a group of sandy soils and 12.3 higher with a group of heavier soils than did the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method. With the limed soils of the latter group the $Ba(OH)_2 - NH_4Cl$ method gave values which averaged only 4.4 per cent higher. These results are interpreted to mean that liming, like treating a soil with an alkaline solution, results in a "build-up" or the formation of additional exchange complex. For this reason the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method seems preferable in the determination of total exchange capacity. The percentage base saturation data given in this study were therefore calculated from the total exchange capacity values obtained by the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method and the exchangeable hydrogen values obtained by the barium acetate method.

A study was made of the percentage base saturation of a number of soils of similar and of different origins at like pH values. It was found that soils of the same reaction may vary considerably in their percentage base saturation. In general, it was found that the highly weathered soils, such as those from the Piedmont Plateau and some from the Coastal Plain, have a lower degree of saturation at given pH values than less weathered soils, as those from the Coastal Plain Black Belt and the Glacial and Loessial Province.

No relation was found between the organic matter content of soils or the nature of the bases present in the exchange complex and the percentage base saturation of the soils at like pH values. The silica-sesquioxide ratio and the total exchange capacity of the colloids extracted from the soils showed a general, although imperfect, correlation with the percentage base saturation values of the soils at like pH values.

Determinations were made of the avidity of the soil acids in the different soils at similar pH values. It was found that the avidity or strength of the acids present was quite different for different soils. In general, highly weathered soils were found to have weaker acids than less weathered soils. A good correlation was obtained between the avidity of soil acids and the percentage base saturation of soils at pH 4.80.

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A MATHEMATICAL STUDY OF THE DECREASE OF CROP YIELDS

J. DUDLEY GREAVES

Utah Agricultural Experiment Station[1]

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The factors underlying and controlling the general processes of plant growth and nutrition are understood only to a very limited extent. It is known, however, that in any soil there are plant nutrients which are present in quantities smaller than required for optimum plant growth. The magnitude of the deficiency of any soil nutrient from the concentration required for optimum plant growth will constitute a limiting factor of plant growth in that soil. The most deficient nutrient will thus become a controlling factor of plant growth.

In order to obtain a first approximation of the influence of a deficient element on the crop yield we might make the following assumptions without serious error: (*a*) The rate of increase of crop yield with increase of the deficient element is proportional to the magnitude of the deficiency of the limiting nutrient from an optimum concentration; (*b*) the time rate of depletion of the deficient element, provided none is added from outside sources, is proportional to the product of the content of the deficient element in the soil and the crop yield. These two assumptions lead to the conclusion that the crop yield will diminish from year to year at a rate that will be dependent upon the physiological requirements of the crop for that element and upon the length of time plants needing that element have been grown upon the soil.

Using these assumptions, Greaves and Gardner (2) developed a theoretical formula showing the effect on crop yield when sulfur is the deficient element. Their formula was developed as follows:

Let y = crop yield per annum,
s = soil content of the deficient element,
s_0 = soil content of the deficient element for optimum growth of the crop,
k_1 and k_2 represent proportionality constants as yet undetermined,
t = time.

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We may then express the assumptions in a mathematical form as follows:

$$(A) \qquad \frac{dy}{ds} = k_1 (s_o - s)$$

$$(B) \qquad \frac{ds}{dt} = - k_2 (sy)$$

Integrating (A), we obtain

$$(C) \qquad y = k_1 \left(s_o s - \frac{s^2}{2} \right) + k_3$$

Differentiating with respect to the time

$$(D) \qquad \frac{dy}{dt} = k_1 (s_o - s) \frac{ds}{dt}$$

Eliminating $\frac{ds}{dt}$ by means of (B), equation (D) becomes

$$(E) \qquad \frac{dy}{dt} = - c (s_o - s) sy$$

Where $c = +k_1 k_2$.

Equation (E) will integrate giving

$$ln \frac{y}{y_o} = - c \int (s_o - s) \, sdt,$$

or written in exponential form

$$(F) \qquad y = y_o \, e^{-c \int (s_o - s) \, sdt}$$

If we were to assume the integration constant of equation (C), k_3, to be zero, the crop yield y will approach zero asymptotically. Equation (E) shows that the tangent to the curve of equation (F) will be zero when s is at an optimum value s_o. We can, therefore, assume without completing the integration that the curve of equation (F) will be of the form shown in figure 1. The slope of the curve will be determined by the crop grown.

In order to test further the validity of the foregoing assumptions, the author has taken the Rothamsted experimental data for those plots continually cropped without the addition of manure. Nitrogen in this case is probably the limiting plant nutrient.

The raw data were found to be very erratic, and in order to eliminate a part of this fluctuation, they were smoothed by taking an equally weighted moving average of 5 points. To do this, the first five crop yields were added together and then divided by 5; next the second to the sixth, inclusive, year crops were treated in the same manner. This was continued over the entire span of the experiment. In this manner it was possible to put the data in a rational form without markedly altering the general data curve.

Because of the difficulty involved in handling the exponential integral it was thought advisable as a first attempt to call[2] $-c\int(s_0 - s)\,sdt = -kt$. Thus, we assume $c(s_0 - s)s$ to be a function independent of the time. This assumption will put equation (F) in the form

$$y = y_0 e^{-kt} \tag{G}$$

Taking the logarithm of both sides, equation (G) becomes

$$z = z_0 - kt \tag{H}$$

Where

$$z = ln\ y$$

and

$$z_0 = ln\ y_0$$

Equation (H) being linear it should then be possible, if our assumptions are correct, to plot the logarithm of the yield against the time and get a straight line as a means of evaluating the constant k. This was done on all the available Rothamsted data with good confirmation of the theoretical values, as shown in figures 2–21, inclusive. It is to be noted, however, that all these curves show only a portion of the theoretical curve as plotted in figure 1. This is just as would be expected, for in each case the land selected for these experiments was in a partially rundown condition, having grown crops for many years under imperfect agricultural methods, also even many virgin soils are deficient in some plant nutrients.

Our work has been limited to the Rothamsted plots because of the long period for which accurate data are available for those plots. In this type of work data collected over short intervals of time may be misleading. For example, in figure 17 there is a marked decline during the years 1859–74 over that of the average trend of the data. It is again shown from 1914 to 1925. In this type of experimental work run under natural conditions there are environmental influences such as rainfall and temperature, which will alter greatly

[2] At the suggestion of Dr. Gardner the integration has been completed by eliminating s from equations (C) and (E), giving,

$$\frac{1}{s_0 - \sqrt{s_0^2 - \frac{2y}{k_1}}} + \frac{1}{2\,s_0}\,ln\,\frac{s_0 + \sqrt{s_0^2 - \frac{2y}{k_1}}}{s_0 - \sqrt{s_0^2 - \frac{2y}{k_1}}} = s_0 c t + \frac{1}{s_0}$$

the integration constant having been obtained by taking t equal to zero when s is at the optimum s_0. In effect, however, the approximation method here proposed gives a much simpler equation and, because of the somewhat erratic nature of the experimental data available, as revealed by the graphs, it has not been regarded as a profitable venture to attempt the evaluation of the three constants of this equation, although, it must be admitted, this would be another step toward a better representation of the yield-time relationship.

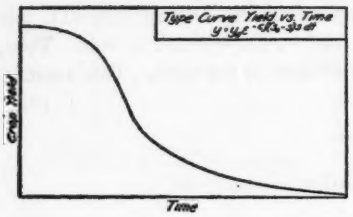

FIG. 1

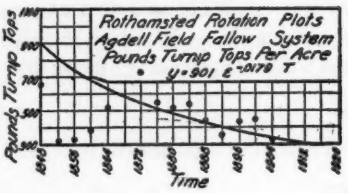

FIG. 2

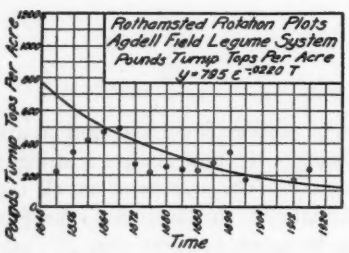

FIG. 3

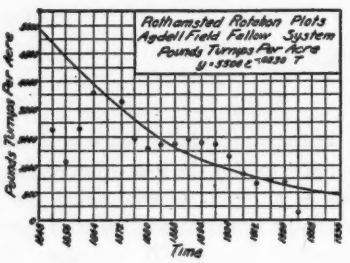

FIG. 4

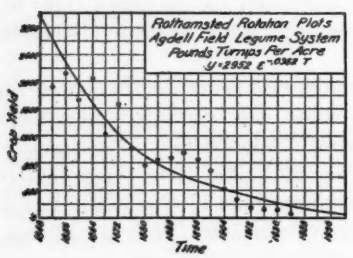

FIG. 5

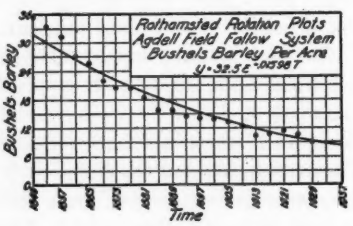

FIG. 6

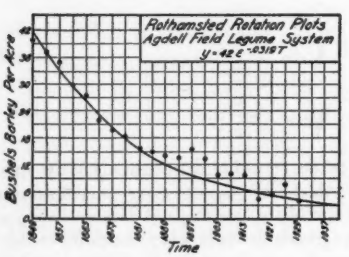

FIG. 7

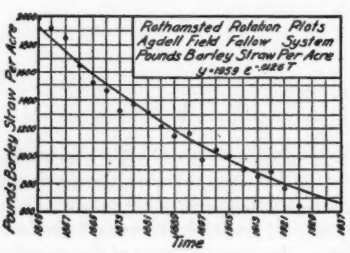

FIG. 8

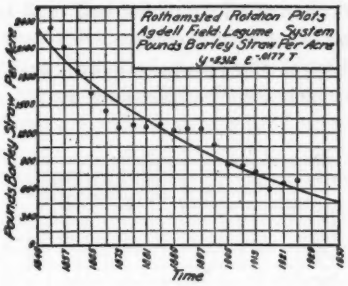

FIG. 9

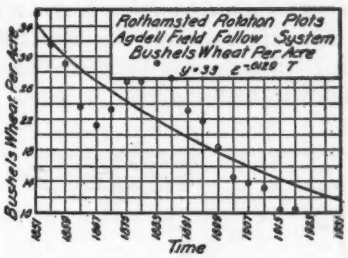

FIG. 10

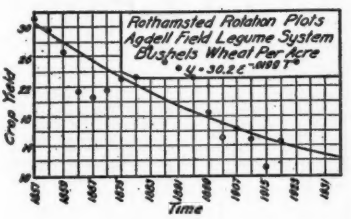

FIG. 11

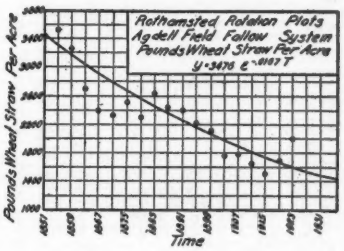

FIG. 12

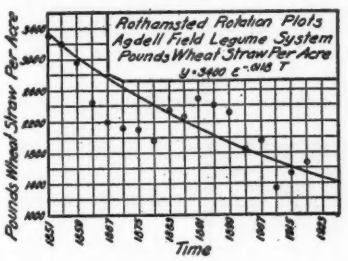

FIG. 13

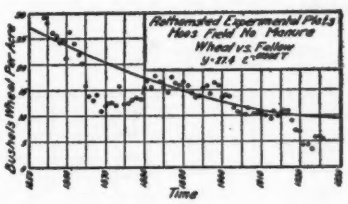

FIG. 14

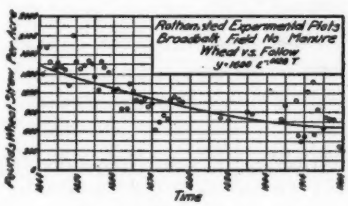

FIG. 15

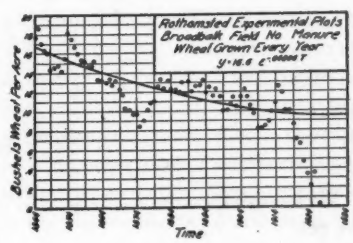

FIG. 16

the individual annual yields; yet when taken over a long period will tend to equalize themselves and so cause a reverting to the natural course.

The data taken from the plots of the Ohio and Illinois stations were considered, but because of the short time over which accurate data were available they have been omitted.

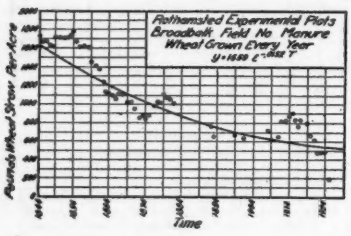

FIG. 17

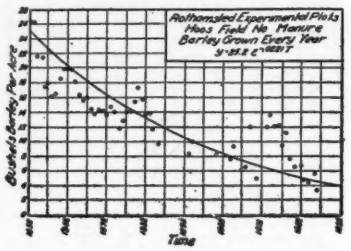

FIG. 18

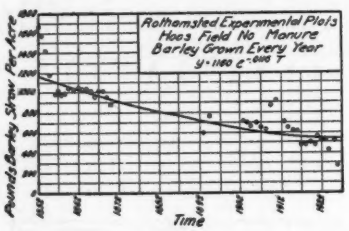

FIG. 19

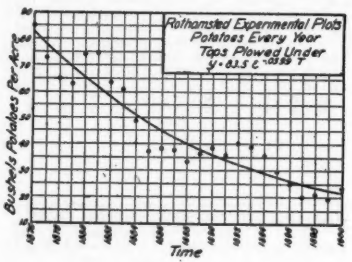

FIG. 20

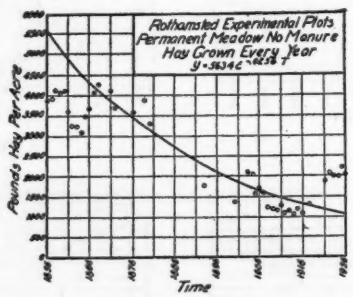

FIG. 21

We have defined our $k = c(s_0 - s)\,s$. Substituting this value in equation (E) we get

$$(I) \qquad \frac{dy}{dt} = -ky$$

It is thus seen that the rate of change of the crop yield with respect to the time is determined by the constant k. We may thus define k as the fractional rate of change of the crop yield from year to year.

It will be noted that in the rotation crop systems carried out on Agdell field the legume system constants are larger than the corresponding fallow system constants. This would tend to show that the use of a legume system of crop rotation will deplete the soil of its nutrients more rapidly than a corresponding fallow system of rotation, as earlier pointed out by Greaves (1).

TABLE 1

Summary of characteristic constants

CROP	PLACE	MAXIMUM	PROPORTIONALITY CONSTANTS
Turnip Tops	Agdell field, fallow system	901.0	0.018
Turnip Tops	Agdell field, legume system	795.0	0.022
Turnips	Agdell field, fallow system	5,506.0	0.023
Turnips	Agdell field, legume system	2,952.0	0.036
Barley Grain	Agdell field, fallow system	32.5	0.016
Barley Grain	Agdell field, legume system	42.0	0.032
Barley Straw	Agdell field, fallow system	1,959.0	0.013
Barley Straw	Agdell field, legume system	2,312.0	0.018
Wheat Grain	Agdell field, fallow system	33.0	0.013
Wheat Grain	Agdell field, legume system	30.2	0.020
Wheat Straw	Agdell field, fallow system	3,476.0	0.011
Wheat Straw	Agdell field, legume system	3,400.0	0.012
Wheat vs. fallow	Hoos field, no manure	27.4	0.014
Wheat vs. fallow	Broadbalk field, no manure	1,600.0	0.013
Wheat continually Grain	Broadbalk field	16.6	0.0081
Wheat continually Straw	Broadbalk field	16.5	0.015
Barley continually Grain	Hoos field	25.2	0.022
Barley continually Straw	Hoos field	1,160.0	0.012
Potatoes continually		83.5	0.060
Hay continually	Permanent meadow	5,634.0	0.026

In the Broadbalk field plots, in which wheat was grown continually, the fractional rate of decrease of straw from year to year was 0.0152, whereas in the rotation crop system on Agdell field it was 0.0107 for the fallow and 0.0118 for the leguminous. This would tend to show that the rate of decrease in crop yield with respect to the time is greater in the one crop system than in the rotation system. This same relationship holds on other similar comparisons, as shown in table 1. The continued growth of small grains does not tend to alter the physical conditions of the soil to the same extent as do crops such as hay and potatoes. This would tend, then, to explain the high constant values obtained for these two crops.

SUMMARY

An attempt was made to test further the equation for crop yield developed by Greaves and Gardner (2).

Their exponential integral was assumed to be a linear function of the time.

The formula was then tested on all available Rothamsted data with good confirmation.

This would tend to establish the two assumptions on which the formula was based:

The rate of increase of crop yield with increase of the deficient element is proportional to the magnitude of the deficiency of the limiting nutrient from an optimum concentration.

The time rate of depletion of a deficient element, provided none is added from outside sources, is proportional to the product of the soil's content of the deficient element and the crop yield.

REFERENCES

(1) GREAVES, J. E. 1918 Does crop rotation maintain the fertility of the soil? *Sci. Mo.* 6: 458–466.

(2) GREAVES, J. E., GARDNER, W. 1929 Is sulfur a limiting factor of crop production in some Utah soils? *Soil Sci.* 27: 445–457.

THE FUNGUS FLORA OF THE SOIL

H. L. JENSEN[1]

State Laboratory of Plant Culture, Lyngby, Denmark

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During the last few decades much research has been devoted to the study of the microscopic fungus flora of the soil, and it is at present a generally accepted view that all soils harbor a fairly definite flora of filamentous fungi, especially Mucoraceae and *Fungi imperfecti.* This fungus flora, which varies somewhat in its composition according to climatic and soil conditions, takes an active part in the microbial decomposition processes in the soil, under certain conditions apparently so energetically as to rival the importance of bacteria.

Most of these studies have been carried out on North American, a fair number also on European, soils. [For review of the very voluminous literature, see the works of Brierley (3) and Waksman (58).] In the Scandinavian countries, Hagem (18, 19) in Norway carried out a masterly study of soil Mucoraceae, and other valuable contributions have been furnished by Sopp (44) and Traaen (47). On the fungus flora of Danish soils the information is very scanty, Müller (36) was among the first botanists to point out that humus soils contain an abundance of fungal hyphae, and that these are of different types in peat and mold soils. Rostrup [see Müller et al. (37)] isolated on agar media from three uncultivated heath soils species of the genera *Oidium, Monilia, Penicillium, Dematium,* and *Hormodendron;* from five heath soils planted with pine trees Rostrup isolated the same species, and, further, *Mucor, Fusicolla, Fusidium, Trichoderma, Citromyces, Pachybasium, Verticillium, Stysanus,* and four sterile mycelia. Later Rostrup (43) isolated from various Danish soils: *Mucor ramannianus, Zygorhynchus vuilleminii, Absidia cylindrospora, Absidia rchidis,* and several species of *Penicillium.* Weis and Bondorff (66) isolated from acid forest soils *Mucor ramannianus,* four *Penicillia,* one *Citromyces,* and one *Sporotrichum.*

This paper represents an attempt to obtain some information about the typical groups of fungi in a series of Danish soils of widely varying character, especially cultivated soils, and about the distribution of fungi in relation to soil conditions, and their importance in certain biochemical soil processes.

[1] Now bacteriologist to The Linnean Society of New South Wales, Sydney.

The Qualitative and Quantitative Character of the Soil Fungus Flora

In this study, 100 different soils were included viz., 49 field soils, 6 garden soils, 18 forest soils, 12 moor and meadow soils, 5 heath soils, 5 marsh soils, and 5 other uncultivated mineral soils. Some samples were taken for this specific purpose, and the rest were soil samples forwarded to the laboratory for chemical analysis or lime requirement determination. The samples were examined as soon after arrival as circumstances would permit, and all numbers of microorganisms were calculated on the basis of fresh, moist soil. The following three methods were used: Microscopical examination (9), direct isolation of fungi (49), and plate countings.

MICROSCOPICAL EXAMINATIONS

The microscopical examinations were carried out with only 30 soils, but confirmed fully the statement of Conn (9): mineral soils were more or less poor in mycelium, though the acid soils seemed to harbor more than the neutral and alkaline ones. Of the humus soils the neutral and alkaline ones were not richer in mycelium than the mineral soils, but an abundance of mycelium was observed in the distinctly acid humus soils, especially those of peaty character. As already pointed out by Müller (36), mostly coarse, dark hyphae predominated in these soils; but it is noteworthy that a good deal of this mycelium was apparently represented by dead material—short fragments with frayed cell walls and apparently devoid of protoplasm; it might seem as if the abundance of mycelium is partly due to a defective power of these soils to decompose dead fungus mycelium. An exception was shown by a few samples of sphagnum-peat (samples 74 and 75, table 1), which in spite of their extremely acid reaction appeared very poor in mycelium. This is in agreement with the very small numbers of fungi shown by plate counts from these soils, and is probably connected with their poverty in mineral nutrients.

DIRECT ISOLATIONS

A number of experiments were made by the method suggested by Waksman (49) for distinguishing between spores and vegetative mycelium in the soil—inoculation of agar plates with lumps of soil and isolation of the fungi developing after 24 hours. The agar medium was that described in the following (p. 127), and 45 different soils were tested: 18 field soils, 15 forest soils, 4 moor soils, 4 heath soils, 2 marsh soils, and 2 uncultivated mineral soils. Fungus hyphae were found radiating from the soil in all cases after 24 hours' incubation at 25°C. The fungi were constantly Mucoraceae or Trichodermae, often both groups. They showed the following distribution within the different soil groups:

	times
From *18 field soils* isolated:	
Mucor hiemalis (?)	4
Zygorhynchus spp.	7
Absidia cylindrospora	5
Sterile Phycomycetous mycelium	7
Trichoderma spp.	2
From *15 forest soils:*	
Mucor hiemalis (?)	1
Mucor circinelloides (?)	1
Absidia cylindrospora	2
Sterile Phycomycetous mycelium	3
Trichoderma spp.	11
From *4 moor soils:*	
Mucor spinosus (?)	2
Sterile Phycomycetous mycelium	1
Trichoderma spp.	4
From *4 heath soils:*	
Absidia orchidis	1
Trichoderma spp.	4
From *2 marsh soils:*	
Zygorhynchus sp.	1
Absidia cylindrospora	2
From *2 other soils:*	
Mucor hiemalis (?)	1
Zygorhynchus spp.	2
Trichoderma sp.	1

We see here that the fungi are largely the same as those obtained by Waksman (49) by direct isolation, and further that the soils fall into two well-defined groups; namely, field soils, marsh soils, and uncultivated mineral soils, which yield mostly Mucoraceae, especially *Zygorhynchus Absidia*, and a peculiar sterile Phycomycetous mycelium (*Cunninghamella elegans ?*); and forest, moor, and heath soils, of which the Trichodermae are characteristic. Since the soils of the latter group are, on an average, of a more acid reaction than those of the former, it might be imagined that this difference is due to the reaction, and that the Mucoraceae occur in an active state mostly in less acid, in neutral, or in alkaline soils, the Trichodermae in more acid soils. It is, however, not so certain that the fungi obtained by this method are the only ones present in the mycelial state, since they are all distinguished by their rapidly spreading growth and would easily depress more slowly growing forms. A point which greatly supports this view is that the slowly developing *Mucor ramannianus* occurs with so perfect constancy in all forest soils that one can hardly imagine it occurring only as spores; yet it has never been obtained by direct isolation. It is thus not easy to find an explanation for the difference between the two groups of soils; perhaps the fungi found by direct isolation are those which have the largest amount of mycelium in the soils in question. This would be in agreement with the fact that the Trichodermae, is as shown subsequently, display their greatest activity in distinctly acid soils.

PLATE COUNTINGS

Counts of fungi were carried out in all 100 solis according to the method outlined by Waksman (52), with some slight modifications.[2] The following

[2] The suggestions of Brierley et al. (4) for standardizing the technique in quantitative work with soil fungi unfortunately could not be used to advantage here, since the experimental part of this work was finished before the publication of Brierley's paper. The same is true of the method of McLennan (32) for distinguishing quantitatively between fungus spores and mycelium in the soil.

TABLE 1

Numbers of fungi, bacteria and actinomyces in soils of different character

NUMBER	CHARACTER OF SOIL	pH	FUNGI, PER GRAM	BACTERIA + ACTINOMYCES PER GRAM
			thousands	*millions*
	Field soils			
1	Sand	4.83	123.4	.....
2	Sand	5.15	135.4	.....
3	Sand	8.10	127.0	.....
4	Sand	7.22	92.4	13.4
5	Sand	6.59	218.0	12.0
6	Sand	6.91	166.6	21.6
7	Sand	7.03	41.4	15.7
8	Sand	6.98	79.0	17.6
9	Sand	6.91	87.2	22.5
10	Sand	7.16	108.6	15.8
11	Sand	6.78	145.6	14.9
12	Light loam	7.32	68.8	16.1
13	Light loam	7.15	100.4	17.6
14	Light loam	7.26	109.4	20.5
15	Loam	6.70	66.6	19.5
16	Loam	6.86	80.0	16.7
17	Loam	6.79	67.0	13.0
18	Light loam	6.30	63.0	.....
19	Light loam	6.36	80.8	.....
20	Heavy loam	6.22	107.9	15.3
21	Heavy loam	7.50	164.4	15.8
22	Heavy loam	7.52	78.0	16.2
23	Heavy loam	6.85	132.0	24.0
24	Sand	6.14	96.4	10.3
25	Sand	6.35	104.8	10.6
26	Sand	6.48	113.4	9.9
27	Sand	6.92	104.4	11.2
28	Sand	7.36	112.0	11.0
29	Sand	7.66	109.6	10.5
30	Sand	5.09	260.8	4.45
31	Sand	6.93	207.4	12.6
32	Sand	7.40	198.6	17.0
33	Light loam	6.72	86.4	.....
34	Light loam	7.86	154.6	.....
35	Light loam	5.92	131.4	7.40
36	Light loam	7.56	65.6	.....
37	Heavy loam	6.74	89.4	.....
38	Clay	4.96	98.0	1.75
39	Sand	5.59	87.2	.....
40	Humus	3.74	453.0	.....
41	Humus	7.08	174.0	25.9
42	Humus	6.09	188.0	19.3
43	Humus	7.20	124.0	7.80
44	Humus	3.76	182.8	0.40
45	Heavy clay	3.97	36.7	1.03
46	Heavy loam	8.35	75.6	11.1
47	Heavy loam	7.02	145.0	3.53
48	Heavy loam	7.23	146.6	10.9
49	Light loam	5.56	257.4	11.5
	Garden soils			
50	Light loam	7.15	252.4	48.3
51	Light loam	7.27	154.8	.....
52	Light loam	7.18	154.0	.....
53	Humas	7.22	176.0	.....
54	Light loam	7.38	460.0	102.4
55	Light loam	7.78	173.2	25.7
	Forest soils			
56	Loam	7.75	166.0	22.3
57	Sand	5.66	40.4	.....
58	Sand	4.83	64.0	.....
59	Sand	4.97	229.2	4.55
60	Sand	5.12	78.0	0.83
61	Humus ("Mould")	6.32	46.0	5.92
62	Humus ("Mould")	5.94	45.6	.....
63	Humus ("Mould")	6.06	52.4	6.40
64	Humus ("Mould")	4.12	165.0	0.67
65	Humus ("Mould")	3.34	108.6	0.17
66	Humus (Peat)	4.32	82.0	0.61
67	Humus (Peat)	3.65	140.0	0.17
68	Humus (Peat)	4.01	174.0	.....
69	Humus (Peat)	4.53	56.0	.....
70	Clay	5.54	190.6	7.10
71	Sand	5.65	99.4	4.40
72	Humus	4.45	385.2	3.91
73	Peat	3.86	176.0	0.32
	Moor and meadow soils			
74	High moor	3.62	34.6	0.095
75	High moor	3.86	33.4	.....

TABLE 1—*Concluded*

NUMBER	CHARACTER OF SOIL	pH	FUNGI, PER GRAM	BACTERIA + ACTINOMYCES PER GRAM
			thousands	*millions*
76	High moor	5.14	62.0	4.64
77	High moor	4.58	220.6	1.06
78	High moor	4.24	75.0	0.58
79	Low moor	5.40	54.0	.....
80	Low moor	5.20	108.0	3.55
81	Low moor	5.64	124.0	3.36
82	Low moor	4.26	206.0	1.42
83	Low moor	7.61	121.2	39.3
84	Mud	4.17	274.6	1.10
85	Mud	6.90	68.6	23.3
	Heath soils			
86	Dry peat	4.40	40.4	.....
87	Dry peat	3.85	183.0	0.21
88	Dry peat	4.10	185.4	0.40
89	Dry peat	4.56	46.6	.....
90	Dry peat	4.39	127.4	1.23
	Salt marsh soils			
91	Very heavy clay	6.82	29.7	8.0
92	Very heavy clay	7.29	26.0	.....
93	Heavy clay	6.12	24.3	8.63
94	Heavy clay	6.29	31.0	.....
95	Very heavy clay	5.12	58.6	7.80
	Various uncultivated mineral soils			
96	Light loam	5.20	76.6	.....
97	Sand	7.55	100.0	21.8
98	Sand	8.04	68.0	.....
99	Sand	7.95	184.0	52.3
100	Light loam	8.08	149.2	57.6

medium was used: dextrose, 20 gm.; asparagin, 2 gm.; KH_2PO_4, 1 gm.; $MgSO_4$, 0.5 gm.; NaCl, 0.5 gm.; $FeCl_3$, 0.1 gm.; agar, 25 gm.; H_2O, 1,000 cc.; pH 3.8–4.0. This medium was found preferable to that used by Waksman, which, probably on account of its peptone content, was found to have a tendency to allow a too abundant development of the Mucoraceae and Trichodermae, so that the more slowly developing fungi may be entirely suppressed. The soil suspensions were prepared by shaking usually 10 gm. of soil with 200 cc. of sterile water for five minutes and transferring 1 cc. to 99 cc. of sterile water, whereby a solution of 1:2,000 is obtained. From this suspension 1-cc. portions were transferred to sterile Petri dishes, and a tube of melted agar, cooled to about 42°C., was added to each. Six or eight parallel plates were prepared and incubated for four or five days at 25°C. When the colonies were counted, a record was made of the relative abundance of the various types of fungi occurring on the plates, and representatives of the predominant forms were isolated. In 73 of the soils determinations of the numbers of bacteria and actinomycetes were also carried out. This also was done according to the method of Waksman (53), by plating on the following medium: dextrose, 2 gm.; casein, dissolved in 10 cc. 0.1 *N* NaOH, 0.2 gm.; K_2HPO_4, 0.5 gm.; $MgSO_4$, 0.2 gm.; $FeCl_3$, trace; agar, 15 gm.; pH 6.5–6.6. A dilution 1:10,000–50,000 was generally chosen for the acid soils and one of 1:100,000–200,000 for the neutral to alkaline soils; this gave in most cases 50 to 250 colonies

to each plate. The counting was carried out after 8 to 10 days' incubation at 25°C. Table 1 gives the results of these counts, together with general characterization of the soils and the pH values, measured by the quinhydrone electrode.

The composition of the fungus flora

The composition the fungus flora showed the following features:

The *Mucoraceae* are a typical group of soil fungi, represented in all the soils examined here. Most frequent were: *Mucor hiemalis*-like form; *Zygorhynchus* sp. (*Vuilleminii* ?); *Mucor ramannianus* which, as previously pointed out by Hagen (18), occurred with perfect constancy in all forest soils, generally accounting for the majority of the colonies here. It was further common in heath and moor soils and not rare in sandy field soils; it occurred in 17 sandy field soils, in 2 humus soils (nos. 42 and 43), and in only one loamy field soil. *Absidia cylindrospora* occurred with great frequency in soils of all types. A peculiar sterile Phytomycetous mycelium, which could not readily be identified with any previously described organism, was found more constantly than any other fungus; it occurred in all soils except 57, 62, 66, 67.[3] Besides these dominating forms several others were found, e.g. *Mucor spinosus* and *Absidia orchidis* in some heath and moor soils, and *Mucor racemosus*, *M. circinelloides* and some other non-identified forms. *Rizopus nigricans*, which according to Waksman (50) is one of the most common soil fungi in North America, was found only once (in 65), and *Rhizopus nodusus*, which has been found very commonly in Norway by Hagen (18), was not met with at all. The numbers of Mucor colonies on plates from field soils rarely amounted to more than 12 to 15 per cent of the total, whereas in the forest soils they often reached 25 to 40 per cent, and in the marsh soils they (especially *Absidia cylindrospora* and the sterile mycelium) were absolutely dominating, occupying 60 to 80 per cent of the total.

The next group of typical soil fungi is the genus *Trichoderma* which was represented in nearly all soils except the marsh soils, where it was found only in the strongly acid soil 95. Only once was a white strain found; all others were green, abundantly sporulating forms, mostly corresponding to the species *koningi* and *lignorum*, but so variable and with so many transition forms that a sharp distinction was impossible. A few less common forms were found, which probably represent separate "species;" one of these agreed well with Waksman's (48) strain III. The numbers of *Trichoderma* colonies on the plates were in most cases very small; only in a few cases did they cover 10 to 12 per cent of the total number, in one case (soil 64) even 30 per cent.

The genus *Penicillium* (including *Citromyces*) was, as might be expected, abundantly represented, and species of these were always the dominating forms in field soils, where they generally accounted for 50 to 60 per cent, sometimes 70 to 80 per cent, of the total number of colonies. In forest, heath, and moor soils, too, they were richly represented although often overshadowed by Mucoraceae and sterile mycelia. Marsh soils were poor in Penicillia; the only soil in which they were found at all was the alkaline marsh soil 92. About 40 strains were isolated, but could not be identified; one of them (*Penicillium* sp. I) was found more frequently and in higher numbers than any other; it seemed closely related to *Penicillium commune*. Also a *Citromyces* form (*glaber?*) was quite common.

The genus *Aspergillus* showed some interesting phenomena. These fungi occurred only sporadically in ordinary uncultivated and field soils. *Aspergillus ochraceus* (?) was found in

[3] On a later occasion a mycelium of this type, isolated from an English field soil, was found fructifying on Conn's glycerine-asparaginate-agar and appeared to be *Cunninghamella elegans* Lendner (28). The appearance of the mycelium from the Danish soils agreed very well with Lendner's description, but fructification was never seen to take place.

meadow soil 86, *Asp. fumigatus* in heath soil 89, and *Asp. globosus* (?) in uncultivated mineral soil 99. All garden soils, on the other hand, harbored *Asp. fumigatus*, and in the two greenhouse soils 53 and 54 this was the dominating form, occupying 35 and 70 per cent of the total numbers of colonies, respectively. The plates from the former soil had also a considerable number of colonies of *Asp. niger*. These findings are quite in agreement with the observations of Waksman (50) and Werkenthin (68), that Aspergilli are more common in soils from hot than from colder climates. Jensen (23) records only one species of Aspergillus from Alaska soil. Sopp (44) states that Aspergilli are rare in soils from Norway, and Traaen (47) records the occurrence but once in 70 mycological analyses of soils from the same country. This is not surprising in view of the fact that the Aspergilli are a group of somewhat thermophilic organisms, thriving excellently at 35 to 40°C., where the Penicillia are hardly able to live. The constant occurrence of *Aspergillus fumigatus* in garden soils of ordinary temperature may be due to its having been introduced with compost dung, where organic matter is usually decaying under aerobic conditions at a high temperature.

The next two groups of fungi seemed characteristic of cultivated soils. The first of these was the genus *Fusarium*, especially *Fusarium orthoceras*, which occurred in all field soils except 40, in the garden soils 51, 52, 55, and in the cultivated moor soils 76 and 79. The only uncultivated soils which harbored this species were the moor soils 80 and 83 and the mineral soils 96, 97, and 98. It is interesting to note that Appel and Wollenweber (1) mention this species as being more common in the soil itself than are the other *Fusaria*. Besides this, about a dozen of less constantly occurring Fusaria were isolated, nearly all from field and garden soils. Most common of these was a rarely fructifying, clamydospore-forming species, possibly *Fusarium falcatum*. Further *Fusarium culmorum* and several strains probably belonging to the variable *Fusarium solani*-group were met with.

The second group was represented by an unidentified *Phoma* species, which occurred in all field soils except three very acid ones, viz., 40, 44, and 45.

Besides these typical soil fungi a large number of species belonging to genera of less constant occurrence and difficult to identify were found. Of these can be mentioned: *Monilia* sp., common in moor and forest soils; *Cephalosporium* sp., common in soils of all types; *Botrytis cinerea*, occasionally found in field and forest soils; *Amblyosporium* sp., found in most forest soils; *Acrocylindrium granulosum*, common in soils of all types; *Spicaria* sp., fairly common in soils of all types; *Acrostalagmus cinnabarius*, found twice in field soils; *Mycogene nigra*, fairly common, especially in field soils; *Hormodendron cladosporioides*, fairly common in soils of very different character; *Sepedonium* sp., *Stachybotrys* sp., *Helminthosporium* sp., *Stemphylium* sp., and *Alternaria* sp., occasionally found in field and uncultivated mineral soils; *Ramularia* sp., fairly common in humus soils; and *Stysanus* sp., found once in field soil. Yeasts were met with occasionally, but they did not seem to be typical soil inhabitants [compare (46)]. Further several sterile mycelia and other not readily identifiable forms were isolated.

Thus upon the whole, the fungus flora observed here appears to be of the same general composition as that found by most other research workers (58). Its leading forms are Mucoraceae, Penicilliae, Trichodermae, and Fusaria, together with a number of species belonging to more sporadically occurring genera, mostly of hyphomycetes. Also the numbers of fungi are of the same general order of magnitude as those found in America by Waksman (54) and Starkey (45).

The numbers of fungi

The numbers of fungi vary, as table 1 shows, within wide limits from 24,300 (in 93) to 460,000 (in 54) per gram of soil. If any relationships exist between

these variations and definite soil characters, we may be justified in assuming that the numbers of fungi found by the plate method have a direct bearing on the abundance of fungi in the soil, although the numbers themselves refer only to the soil suspension and therefore depend largely on the method of preparing this, as shown by Brierley and his coworkers (4). Of such soil characters the following are here considered: physical texture of the soil, the humus content, the soil reaction, and the content of plant food.

The physical character exerts but little influence on the numbers of fungi, apart from the fact that all very heavy clay soils, viz., the five marsh soils 91 to 59 and the field soil 45 show remarkably low numbers of fungi—24,300 to 58,600 to the gram. Light sand soils, on the other hand, often have high numbers, such as soils, 1 to 6 and 30 to 32, in which the fungus flora consists mainly of Penicillia, which were very scarce in the marsh soils. It is quite natural, indeed, that the sand soils with their loose, open structure and good aeration would prove a better medium for the growth and fructification of these comparatively large, aerobic organisms than the heavy, sticky clays, of which the marsh soils further are more exposed to water-logging than any other group of soils.

The humus content does not show any definite relations to the fungus counts. Soils mainly consisting of organic matter, such as the moor soils and forest soils of mold or peat type, have not, upon the whole, higher numbers of fungi than the mineral soils. Several typical organic soils, such as the raw Sphagnum peats 74 and 75 and the forest soils 61 to 63, are even decidedly poor in fungi. Others, such as the forest soil 72 and the mud soil 84, have very high numbers.

The soil reaction is universally known to exert a profound influence on the microflora of the soil, and this is also the case here. It has, ~~until~~ since the work of Ramann et al. (41), been nearly a dogma in soil microbiology that "fungi prevail in acid, bacteria in neutral and alkaline soils." Fischer (14) and Traaen (47) called attention to an occasional abundant occurrence of fungi in neutral and alkaline soils, and recently Waksman (58) has emphasized their activity in such soils.

The data in table 1, when all groups of soils are considered, show not the slightest correlation between the numbers of fungi and the corresponding pH values. Note, for example, the 10 most acid soils, nos. 65, 74, 67, 40, 44, 87, 73, 75, 45, and 68; and the 10 most alkaline soils, nos. 46, 3, 100, 98, 99, 34, 55, 56, 29, and 83.

The table further shows that the numbers of bacteria and actinomyces increase with increasing pH values, as would be expected *a priori*. There is, however, no very strict correlation between the reaction and the bacterial numbers, evidently because these are affected by many other factors besides the reaction. A quantitative expression of this correlation can be obtained by computing the correlation coefficient between the hydrogen-ion concentration and the numbers of bacteria plus actinomyces according to the formula of Branais

$$r = \frac{\Sigma\, dx \cdot dy}{n \cdot \sigma x \cdot \sigma y} \pm m = \frac{1 - r^2}{\sqrt{n}},$$

where r is the correlation coefficient, x and y the two covariates, *in casu* the hydrogen-ion concentration and the numbers of bacteria + actinomyces, dx and dy the individual digressions from the arithmetical mean, Σ the sign of summation, n the number of observations, σx and σy the standard deviations

TABLE 2

Comparison between pH values and ratios of fungi to bacteria + actinomyces in soils from table 1

NUMBER	pH	F:B+A*	NUMBER	pH	F:B+A	NUMBER	pH	F:B+A
65	3.34	0.63	81	5.64	0.037	7	7.03	0.0026
74	3.62	0.37	74	5.65	0.023	41	7.08	0.0067
67	3.65	0.85	35	5.92	0.018	50	7.15	0.0045
44	3.76	0.45	63	6.06	0.0082	13	7.15	0.0057
87	3.85	0.87	42	6.09	0.0092	10	7.16	0.0069
73	3.86	0.55	23	6.12	0.0026	43	7.20	0.016
45	3.97	0.36	24	6.14	0.0093	4	7.22	0.0069
88	4.10	0.46	20	6.22	0.0070	48	7.23	0.013
64	4.12	0.25	61	6.32	0.0078	14	7.26	0.0053
84	4.17	0.25	25	6.35	0.0099	12	7.32	0.0043
78	4.24	0.13	26	6.48	0.012	28	7.36	0.010
82	4.26	0.14	5	6.59	0.018	54	7.38	0.0045
66	4.32	0.13	15	6.70	0.0034	32	7.40	0.012
90	4.39	0.10	11	6.78	0.0098	21	7.50	0.010
75	4.45	0.099	17	6.79	0.0052	22	7.52	0.0043
77	4.58	0.21	91	6.82	0.0037	97	7.55	0.0046
38	4.96	0.056	23	6.85	0.0055	83	7.61	0.0031
59	4.97	0.052	16	6.86	0.0048	29	7.66	0.010
30	5.09	0.059	85	6.90	0.0029	56	7.75	0.0075
60	5.12	0.094	9	6.91	0.0039	55	7.78	0.0067
95	5.12	0.0077	6	6.91	0.0077	99	7.95	0.0035
76	5.14	0.015	27	6.92	0.0093	100	8.08	0.0026
80	5.20	0.030	31	6.93	0.016	46	8.35	0.0068
70	5.54	0.027	8	6.98	0.0045			
49	5.56	0.020	47	7.02	0.045			

* F. fungi; B = bacteria; A = actinomyces.

$\left(\text{calculated according to the usual formula } \sigma x = \frac{\sqrt{\Sigma dx^2}}{n}\right)$ of x and y, respectively, and m the standard error of the final result. It must be noted that these calculations have been made, not on the basis of the pH values, but on the corresponding molar hydrogen concentrations, since we must assume that it is the *absolute* concentration of hydrogen ions and not the logarithmic function thereof—namely, the pH value—which is of importance to the microörganisms living in the soil.

This calculation gives us:

$$r = -0.32 \pm 0.12$$

a negative correlation, which is significant, but not very marked. Although thus the actual numbers of fungi show no, and those of bacteria plus actinomyces only a comparatively small, correlation with the hydrogen concentration, the correlation of this with the *ratio of fungi to bacteria plus actinomyces* is very striking. Table 2 shows that this ratio is about 0.4 to 0.9 in the few soils of pH less than 4. When pH increases from 4 to 6 the ratio drops to 0.01 to 0.02, although with considerable variations. Above pH 6 there is no longer any correlation between pH and F: B + A, which here varies between 0.018 and 0.0026, except in the case of soil 47.

A better picture is obtained if we as before calculate the correlation coefficient between the hydrogen-ion concentration and the ratio F:B + A. This gives us:

In the group from pH 6.06 to pH 8.35: $r = -0.033 \pm 0.22$
In the group from pH 5.56 to pH 8.35: $r = +0.56 \pm 0.097$
In the group from pH 5.09 to pH 8.35: $r = +0.67 \pm 0.074$
In the group from pH 3.34 to pH 8.35: $r = +0.82 \pm 0.040$

There is thus above pH 6 no correlation whatever between hydrogen-ion concentration and F:B + A, but at pH 5.5 the correlation becomes positive and significant, and still more so from pH 5 to pH 3.3. Finally, if we consider the whole material we obtain a correlation so perfect that it is only rarely found in biological phenomena. We may conclude from this, that whereas the actual numbers of fungi and bacteria plus actinomyces depend on many factors, the ratio between these two groups is governed mostly by the hydrogen-ion concentration, except in soils of pH above 6.0, where the actual amounts of hydrogen ions seem to be too small to exert a distinct influence.

It is interesting to compare these result with those obtained by Waksman (54), who determined the numbers of bacteria, actinomyces, and fungi in variously treated plots of widely different pH values, from a permanent fertilizing experiment. The figures of his table 13 (54) show a very definite inverse correlation between pH and ratio of fungi to bacteria plus actinomyces, and a calculation of the correlation between hydrogen-ion concentration and F:B + A gives:

$$r = +0.83 \ (\pm 0.071)$$

i.e. practically the same value as the corresponding figure for the Danish soils.

Waksman's data further show an inverse relationship between pH values and the actual numbers of fungi to bacteria plus actinomyces, which was not observed in the present investigation. A calculation of the correlation between hydrogen-ion concentration and numbers of fungi in Waksman's table gives:

$$r = +0.65 \ (\pm 0.13)$$

The bacteria plus actinomyces, as in our results, increase generally with increasing pH values, and a calculation of the correlation between these numbers and the hydrogen-ion concentration gives:

$$r = -0.60\ (\pm 0.17)$$

These figures are both significant, although the standard error is without significance in cases with so few observations (16).[4]

We see thus that the correlations of actual numbers to hydrogen-ion concentrations are good, but not so perfect as that of the ratio F:B + A, which was also the case with our results. That the actual numbers in Waksman's results were better correlated with the hydrogen-ion concentration than was the case with the Danish soils, is probably because Waksman's figures have

TABLE 3

Influence of lime on numbers of fungi, bacteria, and actinomyces in field experiments

NUMBER OF SOIL	PROVENANCE	LIME PER HA.	pH	FUNGI PER GRAM OF SOIL	BACTERIA + ACTINOMYCES PER GRAM
		tons		*thousands*	*millions*
1	Askov Experiment	0	4.83	123.2	.....
2	Station	0	5.15	135.4	.....
3		32	8.10	127.0	.....
24	Borris Experiment	0	6.14	96.4	10.3
25	Station	2	6.35	104.8	10.6
26		4	6.48	113.4	9.9
27		8	6.92	104.4	11.2
28		16	7.36	112.0	11.00
29		32	7.66	109.6	10.5
30	Tylstrup Experiment	0	5.09	260.8	4.45
31	Station	8	6.93	207.4	12.6
32		32	7.40	198.6	17.0

been obtained from differently treated plots of the same soil, whereas the present data represent many different soils of widely varying character, where a much greater number of uncontrolled factors have been influencing the numbers of microörganisms. If differently treated plots of the same soil are compared, we do sometimes find a decrease in numbers of fungi with an increase of pH. Table 3 shows the data for soils from liming experiments, from which countings have been carried out.

In the Askov experiment, and also in the Borris experiment, where the pH values are all above 6.0, there is no effect of liming on fungi. In the Tylstrup experiment there is a small but noticeable reduction of the fungi, and a very great stimulation of the bacteria and actinomyces due to liming.

[4] According to Fisher (16) more than 100 trials would be necessary to obtain this correlation by random sampling from an uncorrelated population.

To test this point further, a series of liming experiments was carried out in the laboratory. Samples of soils of more or less acid reaction were divided into two parts, to one of which enough $CaCO_3$ was added to make its reaction

TABLE 4

Influence of liming on numbers of fungi in acid soils in the laboratory

SOIL AND TREATMENT	PERIOD OF INCUBATION	pH	FUNGI PER GRAM	BACTERIA + ACTINOMYCES PER GRAMS	ACTINOMYCES	RATIO F:B+A
	days		*thousands*	*millions*	*per cent*	
Heath soil (dry peat) no. 87, no addition	Start	3.85	183.0	0.21	None on	0.87
	15	4.26	494.0	0.72	casein	0.68
	45	3.67	610.0	0.84	agar	0.73
	75	3.90	910.0	1.47		0.62
Same soil + 4 per cent $CaCO_3$	Start	....	183.0	0.21	....	0.87
	15	7.52	420.0	83.1	22.0	0.0051
	45	7.47	393.0	398.0	21.1	0.0010
	75	7.59	595.0	415.0	29.6	0.0014
Low moor soil no. 82, no addition	Start	4.26	206.0	1.42	4.6	0.14
	15	4.10	202.0	1.08	3.1	0.19
	45	4.18	191.5	0.54	7.1	0.36
	75	4.13	223.0	1.16	....	0.19
Same soil + 4 per cent $CaCO_3$	Start	....	206.0	1.42	4.6	0.14
	15	8.05	262.8	60.8	2.6	0.0043
	45	7.50	201.5	96.5	2.5	0.0021
	75	7.76	298.0	161.0	10.9	0.0019
Sand soil no. 30, no addition	Start	5.09	260.8	4.45	40.8	0.059
	15	4.71	341.0	5.14	61.0	0.066
	90	4.42	547.0	4.61	57.6	0.12
Same soil + 1 per cent $CaCO_3$	Start	....	260.8	4.45	40.8	0.059
	15	7.57	365.0	22.9	35.0	0.016
	90	7.50	605.0	43.7	23.4	0.014
Light loam no. 35, no addition	Start	5.92	131.4	7.40	32.0	0.018
	15	6.04	148.0	10.6	24.0	0.014
	50	5.78	126.8	7.87	36.2	0.017
Same soil + 1 per cent $CaCO_3$	Start	5.92	131.4	7.40	32.0	0.018
	15	7.72	72.0	19.2	15.5	0.0037
	90	7.62	120.0	17.1	20.4	0.0055

distinctly alkaline. Enough distilled water was added to saturate it to 70 per cent of its water-holding capacity, and the samples were kept at 25°C. in glass dishes, covered with Petri dish tops, for up to 120 days, during which the moisture content was kept as constant as possible by the addition of dis-

tilled water, and at various intervals of time counts of fungi, bacteria, and actinomyces were made.

The results (table 4) agree well with the previous ones. In the heath soils there is a relative depression of the numbers of fungi: there active multiplication, probably because of the alteration of the structure and the better aeration of the soil, reaches a considerably smaller extent in the limed than in the unlimed soil. In the moor and the sand soil the fungi seem to be stimulated by the liming. In the loam soil, finally, there is first a decrease of fungi in limed soil, later followed by a corresponding increase. The bacteia and actinomyces are strongly affected. Although their numbers do not vary much in the untreated soils, they undergo in the limed soils a rapid multiplication, which in the heath soil—a mixture of coarse sand and peat, rich in undecomposed plant residues—is enormous, from 0.2 to 415 millions to a gram of soil. In the two less acid mineral soils their multiplication, although vigorous, is far less pronounced, probably because the amount of microbial food, which the liming renders available to the bacteria, is smaller in these soils, where a fairly abundant bacterial flora has been active before the liming.

The last column of the table shows a ratio of F:B+A of quite the same order of magnitude as in soils of corresponding reaction taken directly from the field. The agreement with the natural conditions is indeed so good that when the coefficient of correlation between hydrogen-ion concentration and F:B+A in the 24 counts[5] presented in table 4 is calulated, we obtain:

$$r = 0.81 \pm (0.07)$$

This shows that the abundance of fungi, in contact to that of bacteria and actinomyces, as determined by the plate method, is not much influenced by liming of the soil. One gets the impression that the fungi as a whole do not prefer acid reaction, although it is a commonplace that they are much more independent of the reaction of the medium than are the bacteria and actinomyces. Experiments were made to determine the ability of some common soil fungi, actinomyces, and bacteria to grow at different hydrogen-ion concentrations. This is a field in which we possess definite knowledge for only a comparatively small number of soil organisms, since most research in this direction has been devoted to technically and pathologically important microorganisms rather than to ordinary soil saprophytes.

A number of fungi were grown in the following medium: dextrose, 10 gm.; asparagine, 1 gm.; KH_2PO_4, 2 gm.; $MgSO_4$, 0.5 gm.; NaCl, 0.5 gm.; $FeCl_3$, 0.1 gm.; H_2O, 1,000 cc. Portions of this solution were titrated by means of hydrochloric acid and sodium hydroxide to pH values ranging from 1.2 to 8.8. The solutions were then distributed in 10-cc. portions to Jena glass test tubes and sterilized, whereupon the reaction was measured again. Duplicate cultures were incubated at 25°C. until no further change in the amount of growth was observed. Table 5 gives the results.

[5] The counts at the start are common for limed and unlimed soil.

The fungi show a considerable difference in their tolerance toward acidity. The extremes are represented by *Penicillium* sp. I and *Trichoderma* sp. V, which still grow at pH 1.4, and by *Coccospora* sp. (mentioned later) which fails to grow between pH 3.7 and 4.3. A distinct optimum at acid reaction is seen only in *Mucor ramannianus*, *Absidia cylindrospora*, and *Trichoderma* sp. V. One of the Fusaria shows some indication of a double optimum, as observed by Hopkins (20) and Lundegårdh (29). It is further seen that all fungi except *Mucor ramannianus* are able to start growth at pH 8.8, a degree of alkalinity exceptional in Danish soils; pH 3.1 all fungi except *Coccospora* and *Phoma* sp. are able to grow (it is noteworthy that the few field soils in which the markedly acid-sensitive *Phoma* was not found, were the strongly acid soils

TABLE 5

Growth of various soil fungi at different reactions

ORGANISM	GROWTH AT pH:													
	1.16	1.42	2.01	2.53	3.83	3.10	3.71	4.27	5.52	6.02	6.57	7.19	7.86	8.8
Mucor hiemalis	0	0	0	2	2	3	3	4	4	4	4	4	4	4
Mucor ramannianus	0	0	0	1	...	2	...	3	...	3	3	2	1	0
Zygorhynchus sp.	0	0	0	0	...	3	3	3	...	3	3	2	2–3	1–2
Sterile Phycomyc.	0	0	0	0–1	...	1–2	...	3	...	3	3	3	3	2
Absidia cylindrospora	0	0	1	5	5	5	5	5	5	4	3	3	3	3
Trichoderma I.	0	0	0	0–1	...	...	2	2	...	2	3	3	2	2
Trichoderma V.	0	1	3	3	3–4	4	5	5	4	3	3	3	3	3
Penicillium sp. I.	0	1	2–3	2–3	...	...	...	3	3	3	3	3	3	3
Citromyces sp.	0	0	2	3	3	4	5	5	5	5	5	5	5	5
Aspergillus fumigatus	0	0	1	1	4	4	5	5	5	5	5	5	5	4
Coccospora agricola	0	0	0	0	0	0	0	2	4	4	4	4	4	4
Mycogone nigra	0	0	0	0	0	1–2	...	3	...	3	3	3	3	2
Fusarium orthoceras	0	0	1	1–2	1–2	2	...	3	...	4	4	3	3–4	3–4
Fusarium (falcatum?)	0	0	0	0–1	1–2	2–3	2–3	5	3	4	5	5	5	5
Phoma sp.	0	0	0	0	0	0	1	2	3	3	3	3	3	3

The growth here and in the following tables is indicated by the characters: 0 = no growth, 1 = trace, 2 = scant, 3 = fair, 4 = good, 5 = excellent growth.

40, 44, 45, of pH 3.7 to 3.9), and in this whole interval, within the limits of which the pH values of nearly all Danish soils lie, most fungi grow equally well. This is in good agreement with the results of Johnson (5), who found *Mucor glomerula* able to develop in the interval from pH 3.2 to 3.4 to pH 8.7 to 9.2, and 6 species of *Penicillium*, *Aspergillus*, and *Fusarium* would grow within the limits of pH 1.6 to 2.2 to pH 9 to 11.2. Within these limits there was a very broad optimal zone which had its greater extent on the acid side of the point of neutrality. Similar figures are given by Hopkins (20), McInnes (31) and Lundegårdh (29) for *Fusaria* (maximal acidity pH 2.4 to 3), by Currie (10) for *Aspergillus niger* (pH 1.4 to 1.6), and by Meacham (34) for wood-destroying basidiomycetes (pH 1.7 to 2). Melin (35) found the

saprophytic soil fungi *Rhizoctonia silvestris* and *Mycelium radisis atrovirens* able to grow equally well at pH 3.4 and 7. Some parasitic and symbiotic fungi behave differently; Melin (35) thus found mycorrhiza-forming species of *Boletus* growing best in pure cultures at pH 5, with a distinct decline on both sides. Weis and Nielsen (67) found that *Polyporus radiciperda* has a distinct optimum at pH 4 to 4.5 and produces only a very slight growth at pH 5.9 to 6.1. We thus get the general impression that the great majority of ordinary saprophytic soil fungi are quite tolerant toward the reaction of the medium,

TABLE 6

Growth of soil actinomyces at different reactions

ORGANISM	GROWTH AT pH:					
	7.34	5.98	5.40	5.16	4.88	4.41
Actinomyces griseus	4–5	5	5	4	0–3	0
Actinomyces griseoflavus	3–4	4–5	5	5	1–2	0
Actinomyces cellulosae	4–5	4–5	4–5	4–5	1	0
Actinomyces fuluissimus	2	2–3	3–4	5	4	3–4
Actinomyces violaceus-ruber	3–4	4–5	3–4	3–4	3–4	0–3
Actinomyces diastatochromogenus	...	5	2	2	0	0
Actinomyces pheochromogenus	2–3	2	2	2	1	0

TABLE 7

Growth of 10 soil bacteria in broth of varying reaction

ORGANISM	GROWTH AT pH:									
	2.58	3.58	3.88	4.08	4.36	4.58	4.78	5.26	6.06	7.27
Bac. mycoides	0	0	0	0	0	0	0	2	4	4
Bac. megatherium	0	0	0	0	0	0	1–2	1–2	2	3
Bacillus III	0	0	0	0	0	0–1	1	1	1	1
Bacillus IV	0	0	0	0	0	0	0	1	3	2–3
Bact. coeruleum (?)	0	0	0	0	0	0	0	2	4	4
Non-spore-forming rod I	0	0	0	0	0	0	0	0	2	2
Non-spore-forming rod II	0	0	0	0	3	4	4	4	4	4
Non-spore-forming rod III	0	0	0	0	3	4	4	4	4	3
Non-spore-forming rod IV	..	0	0	0	0	0	0	1	3	4
Mycobacterium sp.	0	0	0	0	0	0	0–1	4	3–4	2–3

and their preference for acid reaction is not always clearly pronounced. It is therefore not surprising that no direct relationship can be traced between their abundance and the soil reaction.

Quite reverse features are presented by the *actinomyces* and *bacteria*. Seven strains of common soil actinomyces were grown in the following medium: soil extract (1 kgm. of soil + 1 liter of tap water heated for 30 minutes, in the autoclave) 1,000 cc.; soluble starch, 10 gm.; asparagin, 2 gm.; K_2HPO_4, 2 gm.; reaction varied by means of HCl to pH values between 4.4 and 7.4. The solution was used in 5-cc. portions in test tubes, and duplicate cultures were

incubated for 14 days åt 25°C. The results (table 6) confirm those of Waksman and Joffe (59) and Waksman (55), and of the author in another paper (24): most of the typical soil actinomyces are checked in their growth at pH 4.8 to 5.1, although a few grow at pH 4.4 and still lower.

A number of soil bacteria were isolated at random from various soils in table 1, and tested for their resistance to acidity in the following medium: meat extract, 5 gm.; peptone, 10 gm.; glycerin, 10 gm.; sodium citrate, 4 gm.; K_2HPO_4, 2 gm.; H_2O, 1,000 cc. After being clarified, the medium was titrated to pH values ranging from 2.6 to 7.3. The results are presented in table 7 and show plainly that the growth of the majority of the bacteria is checked in the pH interval 4.8 to 5.7. Only two bacteria from acid soil (nos. 60 and 87) continue to grow at pH 4.36. These results are much the same as found by several other investigators for a number of soil bacteria, as shown in the following:

Organism	Maximal acidity pH	Reference
Azotobacter chroococcum	5.6–6.0	Various authors cited after Waksman (58)
Rhizobium radicola	4.3–5.0	
Rhizobium beijerinck	3.2–3.4	
Clostridium amylobacter	5.7	
Clostridium putrificum	5.8	
Bacillus subtilis	4.2	
Bacterium coli	4.4	
Bacterium stutzeri	6.1	
Pseudomonas pyocyanea	5.6	
Spirochaete cytophaga	5.2–5.7	Dubos (13)
Cellulose decomposing vibrios	6.0–6.5	
Unidentified bacteria from peat: 7 strains	5.0	Itano (21)
Unidentified bacteria from peat: 1 strain	6.0	
Unidentified bacteria from peat: 1 strain	4.0	
Unidentified bacteria from peat: 1 strain	3.0	

We must bear in mind that the pH limits found in artificial culture do not necessarily apply strictly to the natural conditions in the soil: firstly, because an organism may show a larger range of tolerance to acidity in its natural habitat, and secondly, because local differences in reaction, perhaps also symbiotic phenomena, may enable an organism to lead an active life in a soil, where the reaction is, *per se*, prohibitive to the development of the organism in question. But still the results show an essential difference between the two groups of soil microörganisms—fungi vs. bacteria and actinomyces—which helps us to account for the interesting correlation between reaction and ratio of fungi to bacteria. Besides the well-known fact that bacteria (and actinomyces) are generally benefited by a neutral to alkaline reaction, we observe that the pH interval 5 to 6 appears critical, or at least unfavorable, to the great majority of soil bacteria and actinomyces. And this is just the region of the scale of reaction where the correlation betwen hydrogen-ion concentration and ratio of fungi to bacteria plus actinomyces becomes significant, as has been shown.

The food supply would of course *a priori* be expected to exert a considerable

influence upon the abundance of fungi. A direct relation between the numbers of fungi in the soils and their fertility in agricultural respect cannot, however, be traced. The fertile loamy soils from Tystofte (nos. 15–17), Aarslev (nos. 22–23), and Lolland (nos. 20–21) have, for instance, lower numbers than the poor sand soils from Askov (nos. 1–11) and Tylstrup (nos. 30–32), and the raw heath soils 37–38. It seems, upon the whole, impossible to compare soils of different character and origin, but if we compare differently treated plots of the same soil, we see a certain influence of fertilization. By collecting the data from soils from fertilization experiments in table 1 we obtain the results given in table 8.[6]

TABLE 8

Influence of fertilization on numbers of microörganisms in field experiments

SOIL NUMBER	PROVENANCE	TREATMENT	FUNGI, PER GRAM OF SOIL	BACTERIA + ACTINOMYCES PER GRAM
			thousands	*millions*
4	Askov Experiment	Unmanured	92.4	13.4
5	Station, Sand Field	Mineral fertilizer	218.0	12.0
6		Farmyard manure	166.6	21.6
7		Unmanured	41.4	15.7
8		Mineral fertilizer	79.0	17.6
9		Farmyard manure	87.2	22.5
12	Askov Experiment	Unmanured	68.8	16.1
13	Station, Loam	Mineral fertilizer	100.4	17.6
14	Field	Farmyard manure	109.4	20.5
18	Lyngby Experiment	Unmanured	63.0	....
19	Station	Farmyard manure	80.8	....
33		Unmanured	86.4	....
34		Farmyard manure	154.6	....
22	Aarslev Experiment	Unmanured	78.0	16.2
23	Station	Farmyard manure	132.0	24.0

There is here a significant increase in numbers of fungi (besides in bacteria and actinomyces) with the addition of fertilizer and especially farmyard manure, as previously found by Fischer (15) and Waksman (54). Besides this, there is another way which enables us to trace the connection between the abundance of fungi and the food supply of the soils, viz., the calculation of the correlation between numbers of fungi and bacteria plus actinomyces. We may assume that the soils with a higher supply of available microbial food will, other things equal, show a higher number of bacteria than soils with a lower supply; and the numbers of bacteria determined by the plate method is a

[6] Some of these data have been previously published in a paper by Christensen (7), together with other counts of bacteria in soils from fertilizing experiments.

reliable index of the density of the bacterial population (16), although it does not give the absolute figures.

If we select the 45 soils with pH values above 6 (where the reaction does not markedly influence the numbers of bacteria and actinomyces) from table 1 and calculate the correlation coefficient between numbers of fungi and bacteria plus actinomyces, omitting the somewhat unusual garden soil 54 with its extraordinarily high numbers of both fungi and bacteria, we obtain:

$$r = +0.41 \pm 0.13$$

This value, which is not very high but which is significant, shows fairly clearly that the factors which favor the existence of high numbers of bacteria and actinomyces in the soil, have a general tendency also to favor the high numbers of fungi. Had soil 54 been included in the calculation, the correlation would have become still better.

It is interesting to compare this result with some data given in a recent paper by Starkey (45), who determined the abundance of fungi, bacteria, and actinomyces at different time in planted and unplanted field and greenhouse soils. The field soil was a neutral loam, and counts were made at five periods in fallow soil and under eight different crops. These 45 counts give us the following correlation between numbers of fungi and bacteria plus actinomyces:

$$r = +0.43 \pm 0.12$$

practically the same as was found here. The greenhouse soil was a heavy clay of pH 5.5. Counts were made at six periods in fallow soil and under five different crops. These 36 counts give:

$$r = +0.32 \pm 0.15$$

a correlation which is just noticeable, since it would, according to Fisher (16), be obtained by chance once in about 20 trials.

The Importance of Fungi in Various Decomposition Processes

The data obtained by the simple plate method have only a limited value and do not allow a comparison between the actual amounts of fungus mycelium in the soils; they should therefore, if possible, be combined with other experiments. The important thing is not merely what numbers of fungi can we obtain from the various soils, but also, to what extent are the fungi capable of multiplying, when some process of decomposition is going on in the soil? To obtain an answer of this question it would be of interest to see how different treatments affect the fungus and bacterial flora in different soils, such as has previously been done by Waksman and co-workers (60, 61, 64) in studies of partial sterilization of soils and decomposition of organic matter. It is this principle, which Winogradsky (69) has introduced methodically as *dynamic microbiology*.

The experiments were carried out in the same manner as the liming experiments mentioned above. The following substances were tested: Nitrogen-free compounds, dextrose and cellulose; nitrogenous materials, casein and alfalfa seed meal.

DECOMPOSITION OF NITROGEN-FREE COMPOUNDS

Dextrose decomposition experiments were carried out with the following soils:

1. Low moor soil from Tylstrup, pH 4.8
2. Forest sand soil from Lyngby, pH 5.1
3. Loamy field soil from Lyngby pH 5.9
4. Sand soil from Askov, pH 6.8
5. Sand soil from Borris, pH 7.7

Dextrose was added in a concentration of 0.5 per cent on a basis of air-dry soil, and enough distilled water to saturate 75 per cent of the water-holding capacity was added. Counts were carried out after 6 and 12 days at 25°C.

TABLE 9

The multiplication of fungi and bacteria in soils of different reactions with addition of dextrose

SOIL	FUNGI PER GRAM			BACTERIA + ACTINOMYCES PER GRAM		
	Start	6 days	12 days	Start	6 days	12 days
	thousands	*thousands*	*thousands*	*millions*	*millions*	*millions*
Low moor soil, pH 4.78.....	124.0	4,560.0	30,280.0	0.55	5.86	7.60
Sandy forest soil, pH 5.07.....	60.0	2,850.0	8,340.0	1.50	34.4	16.8
Light loam, pH 5.92.....	131.4	157.0	323.0	7.40	.....	121.0
Field soil, sand, pH 6.78.....	145.6	166.0	.....	14.9	206.5	.....
Field soil, sand, pH 7.66.....	109.6	290.0	225.0	10.5	71.0	92.0

The results in table 9 are quite striking. The neutral to alkaline soils 4 and 5 show a large increase in the numbers of bacteria (but not of actinomyces), and the fungi are but little affected. On the other hand, in the two distinctly acid soils 1 and 2, the fungi increase greatly, especially after 12 days (perhaps spore formation is taking place at this period) but the bacteria and actinomyces multiply only to a small extent. The fungi which multiplied most markedly were *Mucor spinosus* (?) and Penicillia in the moor soil, *Mucor Ramannianus* and Penicillia in the forest soil. These observations agree partly with those of Waksman and Starkey (60), who also found that a dextrose addition stimulates the bacteria greatly in neutral but not in strongly acid soil. Contrary to the case here, they found no marked influence of dextrose on the abundance of fungi in acid soil, probably because a period of incubation of only two days was used. Table 9 further shows the noteworthy fact that this marked change in the multiplication of the fungi occurs in the pH interval 5.1 to 5.9—the region where the influence of reaction on the ratio of fungi to bacteria and actinomyces becomes noticeable, and where most soil bacteria

and actinomyces begin to be unfavorably influenced by the reaction, as has been shown.

Finally an experiment was run to determine whether the sugar was decomposed more rapidly by fungi in a soil of acid reaction or by bacteria in a soil of alkaline reaction. An acid, sandy forest soil, fairly rich in organic matter, was divided into two portions, to which were added distilled water as before, and to one, 1 per cent $CaCO_3$. They were then kept at 25°C. for eight days, after which counts of microörganisms were made. One per cent of dextrose was then added, and determinations of the evolved CO_2 were carried out for six days ar 25°C. by means of the method developed by Petersen (40). At the end of the experiment, counts of microörganisms were again made. The results are found in table 10.

The dominating fungi were Penicillia and *Zygorhynchus* sp. in the unlimed, and *Monilia* sp. in the limed, soil. Since all dextrose had disappeared after

TABLE 10

Decomposition of dextrose in acid soil with and without lime

		SOIL $CaCO_3$	SOIL + $CaCO_3$
Before experiment	Fungi per gram.....*thousands*	109.2	108.0
	Bacteria + actinomyces per gram....*millions*	2.2	34.5
	Actinomyces.....*per cent*	61.1	2.4
	pH.....	4.48	7.40
Total production of CO_2, mgm. per 100 gm. of fresh soil.....		543.1	735.0
After experiment	Fungi per gram.....*thousands*	21,000.0	980.0
	Bacteria + actinomyces per gram....*millions*	56.2	1,268.0
	Actinomyces.....*per cent*	45.6	0.4
	pH.....	5.01	7.27
	Dextrose in soil.....	None	None

six days and the pH values do not indicate any formation of organic acids, it seems that the sugar has been equally rapidly decomposed in both soils, and that the smaller production of carbon dioxide in the acid soil is due to the extensive synthesis of fungus mycelium which has taken place in this soil.

Decomposition of cellulose

There is much evidence that the decomposition of cellulose and related compounds is one of the chief functions of the fungus flora of the soil. Van Iterson (22) and Koning (26) found a large number of fungi able to grow on filter paper, and nearly all later investigators have pointed out the striking ability of these organisms to utilize cellulose [Traaen (47), Daszewska (11), McBeth and Scales (30), Otto (39), Waksman and co-workers (48, 61, 63, 64), Rege (42) and others].

In the present experiments four soils of different character were used.

TABLE 11

Multiplication of fungi in soils with addition of cellulose

SOIL AND TREATMENT	FUNGI PER GRAM OF SOIL						
	0	15 days	30 days	45 days	60 days	90 days	120 days
	thousands	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*
1. Sand soil, no. 11, with 1.0 per cent cellulose, pH 6.78.	145.6	142.0	.....	.....	.....	148.5	106.0
2. Same +0.2 per cent $(NH_4)_2 SO_4$ nitrified, pH 4.82..	101.5	4,085.0	14,400.0	34,600.0	.....	29,500.0	23,700.0
3. = 2, with addition of 2 per cent $CaCO_3$, pH 7.3.....	101.5	334.0	530.0	293.0	.....	410.0	390.0
4. Loam soil, no. 16, with 1.0 per cent cellulose, pH 6.86	80.0	149.2	230.0	245.6	247.0	260.0	173.6
5. Same +0.2 per cent $(NH_4)_2 SO_4$ nitrified, pH 4.56..	61.4	3,850.0	6,420.0	7,110.0	.....	7,000.0	8,400.0
6. = 5, with addition of 2 per cent $CaCO_3$, pH 7.3.....	61.4	450.0	427.0	145.0	158.8	375.0	287.0
7. Sand soil, no. 27, with 1.0 per cent cellulose, pH 6.92.	104.4	261.0	284.0	245.0	205.0	143.5	.....
8. Same +0.2 per cent $(NH_4)_2 SO_4$ nitrified, pH 4.60..	121.4	5,665.0	30,500.0	36,000.0	45,000.0	54,000.0	.....
9. = 8, with addition of 2 per cent $CaCO_3$, pH 7.4.....	121.4	460.0	2,666.0	3,130.0	3,200.0	2,310.0	.....
10. Loam soil, no. 35, with 1.0 per cent cellulose, pH 5.92.	131.4	135.0	460.0	567.0	470.0	353.0	.....
11. Same +0.2 per cent $(NH_4)_2 SO_4$, not nitrified, pH 5.92*.....	131.4	1,600.0	14,500.0	37,700.0	21,2000	26,900.0	.....
12. = 11, with addition of 2 per cent $CaCO_3$, pH 7.2.....	131.4	1,200.0	720.0	795.0	518.0	.....	.....

* Changed to pH 4.8–4.5 during course of experiment.

They received 1 per cent cellulose as ground filter paper, and distilled water to 70 per cent of the water-holding capacity, in some cases also a source of N. By choosing $(NH_4)_2SO_4$ for this, allowing it to nitrify until the soil had reached a pH value of 4.8–5.0, dividing the soil in two portions, and adding $CaCO_3$ to one of them, samples of the same soil with different reactions were obtained. The multiplication of the fungi was then determined during a period of 90–120 days at 25°C. Table 11 gives the result.

We see that the addition of cellulose alone to the soil causes a certain multiplication of the fungi, but much more striking results are obtained when combined N is also added [compare Waksman and co-workers (61, 64)]. In the acid soils, nos. 2, 5, 8, and 11, the fungi multiply enormously and are here represented almost exclusively by Penicillia and Trichodermae. In the neutral and alkaline soils, nos. 3, 6, 9, and 12, the fungi develop vigorously too, although the counts do not run nearly so high as in the acid soils [compare Waksman and Heukelekian (61), and Dubos (12)]. But the most interesting thing is *that quite a different fungus flora arises at neutral to alkaline reaction.* It consists almost entirely of *Mycogone nigra*, *Acrocylindrium granulosum*, and a fungus closely resembling the *Coccospora agricola* described by Goddard (17). All these forms were present in only small numbers or (esp. *Coccospora*) were not noticeable in the original soil flora. It would now be of interest to see how the different fungi utilize cellulose in pure culture. A series of qualitative experiments were carried out by growing soil fungi on cellulose as a strip of pure Swedish filter paper in test tubes with the following solution: NH_4NO_3, 2 gm.; KH_2PO_4, 1 gm.; $MgSO_4$, 0.5 gm.; H_2O, 1,000 cc. Cultures were incubated for 30 days at 25°C. The results in table 12 show that the Mucoraceae cannot utilize cellulose. These results are in agreement with those found previously by Hagem (19) and by Waksman and Heukelekian (63), and with the fact that they were not richly represented in the flora which develops in the soil with addition of cellulose, as also noted by Waksman and Stevens (65). The Hyphomycetes are quite different: here the inability to utilize cellulose is a rather rarely occurring exception (*Monilia* sp., *Aspergillus repens*, *Sepedonium* sp., sterile mycelium II), and it is especially noteworthy that the two fungi which were the most prominent in the counts from neutral and alkaline soils with cellulose, viz., *Mycogone nigra* and *Coccospora agricola*, are among the very strongest cellulose decomposers. The *Mycogone nigra* found here corresponds well to the description of this organism by Jensen (23) and also shows similarity to the genus *Humicola*, which Traaen (47) and Waksman (57) mention as an active cellulose decomposer. Van Iterson (22) and Otto (39) also have examined species of *Mycogone*, which they both mention as the strongest cellulose decomposing organism studied by them. The prevalence of the *Penicillium-Trichoderma*-group at acid, and of the *Mycogone-Coccospora*-group at neutral and alkaline, reactions agrees with what we found in table 5—that the latter fungi are rather sensitive to acidity and grow well at neutral to alkaline reaction, whereas those of the former group are very resistant to acidity and

may show a definite optimum at acid reaction. It also agrees perfectly with the results of Dubos (13), who found *Penicillium* sp. and *Trichoderma koningi* able to grow on filter paper at pH values between 2.1 to 3.0 and 9.0 to 9.5, with an optimum at pH 3.5 to 5.0 for *Penicillium* and 3.06 to 5.5 for *Trichoderma*, whereas *Humicola* sp. (=*Mycogone nigra?*) would grow from pH 3.0 to 9.5 with an optimal zone from pH 3.5 to 8.5.

To determine whether this difference is also apparent in the activity of the two groups of fungi in decomposing cellulose at acid and alkaline reactions, a quantitative cellulose decomposition experiment was carried out. A light

TABLE 12

Growth of various soil fungi on filter paper cellulose

ORGANISM	GROWTH	ORGANISM	GROWTH
Mucor hiemalis	0–1	*Penicillium* XXII	4
M. circinelloides	0–1	*Amblyosporium* sp.	4
M. Ramannianus	0	*Botrytis cinerea*	5
M. spinosus	0	*Monosporium* sp.	4
Zygorhynchus sp.	0–1	*Sepedonium* sp.	0
Absidia cylindrospora	0–1	*Acrocylindrium granulosum*	3
Cunninghamella elegans	0–1	*Coccospora* ?	5
Monilia sp.	0	*Acrostalagmus cinnabarinus*	3
Cephalosporium sp.	2	*Citromyces glaber*	3
Trichoderma I.	4	*Spicaria* sp.	4
Trichoderma II.	3	*Mycogone nigra*	5
Trichoderma III.	1	*Hormodendron cladosporioides*	3
Trichoderma IV.	3	*Stachybotrys* sp.	4
Trichoderma V.	5	*Stemphylium* sp.	4
Aspergillus repens	0	*Stysanus stemonites*	3
A. fumigatus	3	*Fusarium solani*	3
Penicillium I.	4	*F. orthoceras*	2
Pencillium II.	3	*F. culmorum*	3
Pencillium IV.	3	*Fusarium* sp. X.	5
Pencillium V.	3	*Ramularia* sp.	3
Penicillium IX.	3	Sterile mycelium I.	3
Penicillium XV.	3	Sterile mycelium II.	0
Penicillium XVI.	3	Sterile mycelium II.	1
Penicillium XVII.	4	Sterile mycelium IV.	5

sandy field soil, poor in organic matter, from the Tylstrup Experiment Station was used. The soil received—on an air-dry basis—1 per cent cellulose as ground filter paper, and 25 per cent of the following solution: NH_4NO_3, 5 gm.; KH_2PO_4, ½ gm.; *N*-HCl, 8 cc.; H_2O, 1,000 cc. This gave the soil a pH value of 4.5 (originally 4.8). Portions of 120 gm. of moist soil were placed in round 300-cc. flasks stoppered with perforated rubber stoppers, in which the hole was filled with cotton wool. The flasks were then sterilized in the autoclave, and in half of them the soil was made alkaline after sterilization by the addition of sterile $CaCO_3$. They were then inoculated with pure cultures of *Penicillium*

sp. I, *Trichoderma* sp. V, *Mycogone nigra*, and *Coccospora agricola*. For comparison a corresponding experiment was run with unsterilized soil. After 30 days' incubation at 25°C. determinations of residual cellulose by the method of Charpentier (6) showed the results reproduced in table 13 (average of two parallels).

These results are in perfect agreement with the previous ones and with those of Dubos (13): *Penicillium* and *Trichoderma* show a stronger activity at acid reaction, though *Trichoderma* is but little influenced. *Mycogone* and especially *Coccospora* are very distinctly favored by an alkaline reaction. It is further seen that the natural soil flora decomposes the cellulose more rapidly

TABLE 13

Decomposition of cellulose by fungi in acid and alkaline soils

ORGANISM	PER CENT OF ADDED CELLULOSE DECOMPOSED IN	
	Acid soil (pH 4.5)	Alkaline soil (pH 7.6)
Uninoculated	0	0
Penicillium sp. I	62	32
Trichoderma sp. V	80	70
Mycogone nigra	40	78
Coccospora agricola	23	66
Unsterilized soil	85	91

TABLE 14

Fungi, bacteria, and actinomyces in acid and alkaline soil plus cellulose

	FUNGI PER GRAM	BACTERIA + ACTINOMYCES PER GRAM	ACTINOMYCES
	thousands	*millions*	*per cent*
Soil before experiment	41.0	0.97	10.9
Soil after experiment:			
Without $CaCO_3$	1,360.0	8.6	2.3
With $CaCO_3$	178.0	71.4	1.6

than any of the pure cultures, although *Trichoderma* in acid soil and *Mycogone* in alkaline soil are not much behind. It also appears, in agreement with the results of Charpentier (6), Barthel and Bengtsson (2), and Waksman and Heukelekian (61), that cellulose decomposition is approximately equally rapid with and without addition of lime to acid soil.

Counts of microörganisms were carried out in the unsterilized soils before and after the experiment, with the results shown in table 14.

In the acid soil the dominating fungi after the experiment were *Penicillium* sp. XVII (*purpurogenum?*) and *Mucor ramannianus;* in the alkaline soil, *Mycogone nigra* and *Mucor ramannianus*. The results thus agree with the

previous ones, except for the abundant development of *Mucor ramannianus*. It is further seen, in agreement with Waksman and Starkey (60), and Waksman and Skinner (64), that the bacteria and actinomyces are not much affected by the addition of cellulose; the strong multiplication of these organisms in the alkaline soil may simply be due to the action of the lime (compare table 4).

The fungi are thus active in the decomposition of cellulose both at acid and alkaline reactions. Under natural conditions they are probably less important in neutral and alkaline soils, since there they must share the energy material with cellulose decomposing bacteria, which are strongly active under these conditions (12), and the cellulose is not decomposed much more rapidly in this case than at an acid reaction, where the cellulose is at the disposal of the fungi alone. But one thing must be kept in mind: the differences in numbers of fungi in acid and alkaline soils in the experiments mentioned do not indicate corresponding differences in activity, since the abundantly sporulating Penicillia and Trichodermae must necessarily show figures of quite another order of magnitude than *Mycogone* and *Coccospora*, which produce only comparatively few chlamydospore-like conidia.

DECOMPOSITION OF NITROGENOUS MATERIALS

Müntz and Coudon (38), and Marchal (33) observed that soil fungi have a marked capacity of decomposing nitrogenous organic materials with production of ammonia, and Butkewitch (5) first called attention to their marked proteolytic activity. Since then a considerable amount of work (8, 27, and 48) has been devoted to the study of the "ammonifying power" of soil fungi. Waksman (51, 62) pointed out that the accumulation of ammonia is not an index of proteolytic activity and therefore not a fair basis for the comparison of microörganisms of different proteolytic powers. Organisms which produce little ammonia may produce much amino nitrogen, and ammonia accumulation is rather an index of the utilization of nitrogenous compounds as energy material and therefore entirely dependent on both the nature of the microorganisms and the carbon-nitrogen ratio of the decomposed material.

Since the correctness of these arguments was recognized, no "ammonification experiments" were carried out, but an attempt was made to determine the importance of fungi in protein decomposition by adding nitrogenous materials to soils of various character, isolating the fungi which were specially stimulated, and testing their proteolytic activity.

Decomposition of casein

The first set of experiments was carried out with casein as an example of a pure protein. Four soils were used; they received 1 per cent casein, and water to 75 per cent of water-holding capacity, in some cases also 2 per cent cellulose. Counts of microörganisms were made over a period of 45 days. Table 15 shows the results.

The fungi multiply enormously in the two strongly acid soils, and in no. 2

there is an equally rapid decrease after 30–45 days, so that at this period the fungi disappear almost entirely. Still higher and more consistent figures are obtained when cellulose is also added. The composition of the flora is interesting: it consists in no. 1 almost entirely of *Mucor hiemalis*, *Mucor ramannianus*, and sterile Phycomycetous mycelium (*Cunninghamella elegans?*); when cellulose too is added, *Mycogone nigra* appears in large numbers. In no. 2 the flora both with and without cellulose consists of *Mucor ramannianus* and

TABLE 15

Multiplication of fungi, bacteria, and actinomyces in soils during decomposition of casein

SOIL AND TREATMENT		START	AFTER 10 DAYS	AFTER 20 DAYS	AFTER 30 DAYS	AFTER 45 DAYS
1. Sandy forest soil, pH 4.95, + 1 per cent casein	Fungi	236.5	2,360.0	7,040.0	7,325.0	.....
	B + A	4.77	533.2	538.8	510.0	.....
	A. per cent	13.3	2.4	20.2	13.7	.....
1a. Same + 2 per cent cellulose	Fungi	236.5	14,640.0	12,300.0	10,850.0	.....
	B + A	4.77	2,850.0	4,310.0	322.0	.....
	A. per cent	13.3	1.8	2.4	14.4	.....
2. Heavy clay, no. 38, pH 4.96, + 1 per cent casein	Fungi	86.0	27,000.0	8,850.0	.....	37.0
	B + A	1.86	81.5	86.1	765.0	76.7
	A. per cent	22.2	30.9	4.6	3.2	4.8
2a. Same + 2 per cent cellulose	Fungi	86.0	71,000.0	88,000.0	94,600.0	.....
	B + A	1.86	63.5	344.4	242.2	.....
	A. per cent	22.2	35.5	45.2	57.1	.....
3. Light loam, no. 35, pH 5.92, + 1 per cent casein	Fungi	131.4	356.0	837.0	413.0	363.0
	B + A	7.4	246.6	546.0	170.0	45.1
	A. per cent	32.0	1.9	1.0	2.5	5.7
4. Loamy garden soil, pH 8.03, + 1 per cent casein	Fungi	172.0	(0)	4.8	9.0	.....
	B + A	42.6	626.4	1,702.5	178.3	.....
	A per cent	19.3	6.7	0.4	3.6	.....
4a. Same + 2 per cent cellulose	Fungi	172.0	118.0	640.0	870.0	1,018.0
	B + A	42.6	1,798.0	448.0	78.0	105.7
	A. per cent	19.3	2.9	5.4	4.3	7.8

Penicillium spp. In the faintly acid soil no. 3 the multiplication of the fungi is less abundant, and the flora is mostly represented by *Mucor racemosus* and *Fusarium orthoceras*. In the alkaline soil no. 4 the fungi are nearly exterminated by the addition of casein alone; when cellulose too is added, there is a considerable multiplication of fungi, especially the strong cellulose decomposer *Coccospora agricola*, which is so markedly favored by alkaline reaction. The bacteria and actinomyces multiply enormously in the acid soil, but not so much

in the alkaline soils; this is probably because the ammonia production soon gives the acid soils a reaction favorable to the bacteria and actinomyces, but renders the alkaline soil too alkaline. Although thus the fungi are known to be most active in decomposing proteins in acid soils, they may be more or less active under alkaline conditions, especially when cellulosic material is also present, such as will mostly be the case under natural conditions. The Mucoraceae and Penicillia, and in some instances Fusaria, seem to be the most active fungi; *Trichoderma koningi*, which has been shown by several investi-

TABLE 16

Multiplication of fungi, actinomyces, and bacteria in soils during decomposition of alfalfa seed meal

SOIL + 1 PER CENT ALFALFA SEED MEAL		START	AFTER 10 DAYS	AFTER 20 DAYS	AFTER 30 DAYS	AFTER 45 DAYS	AFTER 60 DAYS
1. Sandy forest soil, pH 4.54	Fungi*	247	199,200	72,600	59,400	48,300	56,800
	B + A†	2.5	12.4	16.0	8.4	4.1	3.5
	A. per cent	18.5	.....	.....	.....	23.9	29.2
2. Heavy clay soil, no. 38, pH 4.96	Fungi	86	32,800	109,200	50,300	77,000	108,000
	B + A	1.9	45.0	.....	62.0	26.8	17.4
	A. per cent	22.2	23.7	.....	9.1	26.1	22.8
3. Light loam, pH 5.92	Fungi	131	6,430	5,140	5,300	4,430	5,860
4. Heavy clay soil, no. 38, + 1.0 per cent $CaCO_3$, pH after 10 days 7.48	Fungi	86	2,115	1,218	1,474	2,844	944
	B + A	1.9	230.0	280.7	223.4	217.6	162.0
	A. per cent	22.2	39.1	25.2	36.1	47.7	39.0
5. Loamy garden soil, pH 7.48	Fungi	192	2,135	1,280	1,020	646	350
	B + A	27.8	2,232.0	2,692.0	612.0	301.4	124.0
	A. per cent	24.4	26.3	29.3	21.1	33.6	44.0

* Fungi: 1,000 per gm. of soil.

† Bacteria + actinomyces: Millions per gm. of soil.

gators to produce very large amounts of ammonia from proteins in pure cultures, does not appear to have been of any importance here, although it was present in the original fungus flora of all four soils.

Decomposition of alfalfa seed meal

These experiments were carried out with the same soils and in the same manner as the previous set. The alfalfa seed meal contained 4.72 per cent nitrogen in air-dry matter and was added in a quantity of 1 per cent of air-dry soil. The results in table 16 are even more striking than those of the previous experiment.

In the strongly acid soils the multiplication of the fungi is so enormous that the mycelia for the first few weeks bind the soil together in one compact mass. The dominating species here are *Mucor ramannianus* and *Penicillium* sp. I and in no. 2, also *Mucor hiemalis*. The bacteria and actinomyces are only slightly affected. In the two alkaline soils, on the other hand, the development of the bacteria and actinomyces is enormous, and that of the fungi relatively scanty. Under these circumstances also they take an active part in the transformation of organic matter, as shown by their rising and then falling numbers and the still macroscopically visible development of mycelium. The fungus flora is here of another character than in the acid soils. It consists mainly of *Mucor hiemalis*, *Mucor racemosus*, and sterile Phycomycetous mycelium and, in the originally acid soil no. 4, also of *Penicillium* sp. and *Fusarium orthoceras*. The faintly acid soil represents an intermediate stage as to the multiplication of the fungi. Its flora consists of *Mucor racemosus*, *Mocor hiemalis*, *Penicillium* spp., and *Fusarium orthoceras*. The general aspect of the fungus flora is thus about the same as in the experiments with casein.

Proteolytic power of fungi in pure culture

To determine the comparative proteolytic powers of the various soil fungi, two sets of experiments were carried out.

Proteolytic activity in gelatin. In the first series of these experiments, fungi were grown as ordinary test tube cultures on slopes of 15 per cent "gold label" gelatin in distilled water, with and without 1 per cent dextrose. After 20 days' incubation at 25°C. the gelatin was melted, if not completely liquefied, and determinations were made, in 2-cc. portions, of ammonia-N by distillation with MgO, and of formol-titrating N by the method of Sørensen. The results are given in table 17.

In most cases the presence of dextrose decreases the extent of proteolysis, because the dextrose by acting as energy material saves the protein from attack (51). But it does not hold for the proteolytically strongest organisms, *Citromyces glaber*, *Fusarium orthoceras*, and two organisms included for comparison, viz., *Actinomyces griseus* and *Bacillus mycoides*. Their activity is in some cases actually increased by the presence of the sugar. Among the fungi only, *Fusarium orthoceras* is about equal to *Bac. mycoides* in proteolytic respect, and none of these reach *Actinomyces griseus*.

A somewhat different result was obtained in the second series of experiments. The following medium was used: gelatin, 20 gm.; dextrose (if added), 20 gm.; K_2HPO_4, 2 gm.; $MgSO_4$, 0.5 gm.; NaCl, 0.5 gm.; $FeCl_3$, 0.1 gm.; H_2O, 1,000 cc.; pH 6.4. The solution was used in 100-cc. portions in 350-cc. Erlenmeyer flasks. After incubation for 20 days at 25°C. the cultures were filtered, the mycelia dried and weighed, the filtrates restored to 100 cc., and determinations of ammonia-N and formol-titrating N carried out.

The results in table 18 show some interesting phenomena. In gelatine

solution without dextrose, *Citromyces glaber* is a fairly strong protein decomposer, and *Actinomyces griseus* a very strong one, whereas the four other fungi and *Bacillus mycoides* show very moderate activities. The growth of the fungi was in all cases poor and was limited to a loose, submerged mycelium without fructification. *Act. griseus* grew excellently, producing a heavy, firm, wrinkled pellicle, covered with a thick, dusty layer of conidia and emitting the strong characteristic soil odor. In the dextrose-cultures the activity of *Bac. mycoides* is strongly inhibited, probably because of acid-poisoning, since this organism forms acid from dextrose and had altered the reaction of the medium

TABLE 17

Proteolytic activity of fungi in 15 per cent gelatin with and without dextrose

ORGANISM	GELATIN WITHOUT DEXTROSE			GELATIN + DEXTROSE		
	Liquefaction	NH_3 – N, mgm. per 10 cc.	Formol-titrating N, mgm. per 10 cc.	Liquefaction	NH_3 – N, mgm. per 10 cc.	Formol-titrating N, mgm. in 10 cc.
Mucor hiemalis	Complete	14.2	16.1	About ¾	9.8	10.6
Mucor ramannianus	Very faint	4.2	2.7	None	1.7	1.4
Zygorhynchus sp.	About ½	15.4	15.4	About ½	....	7.1
Cephalosporium sp.	About ½	8.4	14.7	About ¾	5.6	6.3
Trichoderma koningi	Faint	7.0	9.8	Faint	....	7.0
Aspergillus fumigatus	Faint	9.1	11.2	Very faint	4.9	4.2
Penicillium sp. I.	Faint	10.5	9.1	Faint	6.0	5.6
Citromyces sp. I.	Complete	9.8	26.0	Nearly complete	14.0	25.9
Acrocylindrium sp.	About ½	9.5	14.7	About ¾	8.4	15.4
Coccospora agricola (?)	Complete	14.2	16.8	Complete	16.8	18.9
Mycogone nigra	Faint	11.9	9.8	About ½	5.3	2.1
Fusarium orthoceras	About ¾	21.7	27.3	Complete	33.6	48.3
Fusarium sp. II.	About ¾	14.0	12.6	About ¾	16.5	11.9
Actinomyces griseus	Complete	23.8	51.8	Complete	45.5	54.6
Act. diastatechromogenus	About ½	11.2	37.1	About ¾	4.5	24.2
Bacillus mycoides	Complete	16.5	37.1	Complete	23.1	35.7
Control (sterile)	.....	0.0	3.5	.....	0.0	3.5

to pH 5.1. *Act. griseus* is hardly affected, but the fungi (except *Mucor ramannianus*, for which the reaction is probably the limiting factor) are greatly stimulated in their proteolytic activity, so much indeed, that three of them exceed *Act. griseus* in ammonia-formation and one in formation of formol-N. The explanation is probably that dextrose is so infinitely superior to gelatine as energy material for the fungi that their growth (except *Mucor ramannianus*) is greatly stimulated and appears as heavy, folded, abundantly sporulating mats, containing much more protoplasm than in the solution without gelatin, and this again gives rise to a production of much larger amounts of proteolytic

and de-amidizing enzymes. That the proteolytic activity of fungi is inhibited by the presence of non-nitrogenous energy material may thus be true only *relatively*, that is, when we compare equal amounts of synthesized mycelium, such as shown in columns 4 and 8 of table 18, where the amounts of formol-titrating N are taken as indexes of the proteolytic activity.

TABLE 18

Proteolytic activities of fungi in 2.0 per cent gelation solution with and without dextrose

ORGANISM	SOLUTION WITHOUT DEXTROSE				SOLUTION + DEXTROSE			
	NH_4-N, mgm. per 100 cc.	Formol-titrating N, mgm. per 100 cc.	Weight of dry mycelium, gm.	Formol-titrating N, mgm. per gm. of mycelium	NH_4-N, mgm. per 100 cc.	Formol-titrating N, mgm. per 100 cc.	Weight of dry mycelium, gm.	Formol-titrating N, mgm. per gm. of mycelium
Mucor ramannianus	15.3	21.0	(trace)	....	3.5	13.6	(trace)	....
Mucor racemosus	13.3	48.3	0.18	218	101.2	100.1	0.85	106
Penicillium sp. I.	17.9	38.5	0.20	147	45.5	58.1	0.90	54
Citromyces glaber	67.9	101.1	0.18	505	148.4	144.9	0.65	207
Fusarium orthoceras	17.5	40.6	0.19	166	90.0	107.8	0.95	102
Actinomyces griseus	72.1	135.1	0.20	630	71.7	130.9	....	....
Bacillus mycoides	23.5	56.7	....	....	5.8	23.8		
Sterile solution	0.0	9.1	....	....	0.0	9.1	....	....

TABLE 19

Proteolytic activity of fungi in acid and neutral peptone solution

ORGANISM	ACID SOLUTION			NEUTRAL SOLUTION		
	NH_3-N, mgm. per 50 cc.	Formol-titrating N, mgm. per 50 cc.	pH	NH_3-N, mgm. per 50 cc.	Formol-titrating N, mgm. per 50 cc.	pH
Mucor hiemalis	21.8	23.0	....	14.4	22.3	....
Mucor ramannianus	13.7	30.4	7.43	6.1	18.5	7.26
Trichoderma koningi	16.0	22.7	7.72	10.1	15.7	8.30
Aspergillus niger	41.1	40.3	6.36	41.2	40.3	4.86
Penicillium sp. I.	20.6	24.2	7.92	16.8	30.8	8.03
Citromyces sp. I.	49.1	49.9	....	49.1	50.7	....
Mycogone nigra	18.3	20.9	8.32	....	17.4	....
Fusarium orthoceras	19.6	20.2	8.8	16.8	20.2	9.0
Actinomyces griseus	No growth			13.5	34.5	9.0
Bacillus mycoides	16.4	9.0	6.85	18.5	28.6	8.14
Control (sterile)	1.1	6.2	5.03	1.1	6.5	7.0

Proleolytic activity in peptone. These experiments were carried out in solutions of acid and neutral reaction. The following medium was used: Peptone, 20.0 gm.; K_2HPO_4 or KH_2PO_4, 2.0 gm.; $MgSO_4$, 0.5 gm; NaCl, 0.5 gm; H_2O, 1000 cc. The reaction after sterilization was pH 4.9 with KH_2PO_4, pH 7.05 with K_2HPO_4. The solutions were used in portions of 20 cc. in 50-cc.

Erlenmeyer flasks. Cultures were incubated for 20 days at 25°C. and analysed as above. The results are found in table 19.

It is seen that the two strongest protein decomposers, *Citromyces glaber* and *Aspergillus niger*, are fairly indifferent toward changes in the reaction, whereas acid reaction is favorable to the rest of the fungi, especially *Mucor ramannianus*. Butkewitch (5) found that the proteolysis by fungi in acid solution is carried largely to the ammonia-stage. In the present experiment there is an indication of this only in the case of *Penicillium* sp. I. As might be expected, *Bact. mycoides* and *Act. griaseus* show only slight or no activity in acid solution, and even in neutral solution their activity is not greater than that of several fungi.

These experiments indicate that the extent of proteolysis by fungi is very variable and depends, among other things, on the nature of the protein, the presence of carbonaceous food, and the reaction of the medium, but is often comparable to or may exceed that of *Bac. mycoides* and *Act. griseus*—two soil organisms known to possess particularly strong proteolytic properties.

It might now be asked whether any relation exists between the proteolytic powers of the fungi in pure culture and their activity in protein decomposition in the soil, as indicated by their multiplication. It will be noted that two of the strongest protein decomposers—*Asp. niger* and *Citr. glaber*—appear to play no rôle, since the former does not occur in normal soils, and the latter, although of common occurrence, is not prevalent in the flora developing during the decomposition of casein or alfalfa seed meal in the soil. This is the case with *Mucor hiemalis*, *Mucor racemosus*, *Penicillium* sp. I, and *Fusarium orthoceras*, of which especially the last has shown strong proteolytic properties. A special position is occupied by *Mucor ramannianus*, which multiplies vigorously in acid soil during decomposition of protein and is capable of exerting strong proteolytic activities in acid media, but is greatly inhibited by neutral or alkaline reaction.

GENERAL CONCLUSIONS

The experiments with addition of organic matter to the soil show better than the counts from soils in their natural state the influence of soil reaction on the microflora. Whereas the plate counts show that the ratio of fungi to bacteria plus actinomyces is governed principally by the hydrogen-ion concentration, which exerts its most pronounced influence in the pH interval from 5 to 6, the decomposition experiments show that the presence of decomposable organic material tends to stimulate the activities of fungi under acid and, to a lesser extent, alkaline conditions. Here again, the change in the preponderance of fungi over bacteria plus actinomyces, and vice versa, is marked in the pH interval from 5 to 6, as is shown by the experiments with dextrose, casein, and alfalfa seed meal. This change is hardly due to any specific sensitiveness of most soil fungi to alkalinity, but rather to the sensitiveness of most soil bacteria and actinomyces to acidity. It is probably from this stimulating effect

of organic matter on both the main groups of soil microörganisms, that the positive correlation between numbers of fungi and bacteria plus actinomyces results. This effect depends not only on the hydrogen-ion concentration, but also on the quality of the organic compounds, of which dextrose and casein stimulated only the bacteria in the alkaline soil, whereas the cellulose stimulated the fungi markedly and had but a slight and probably only secondary effect on the bacteria and actinomyces developing in plate counts. The fact that there is a definite correlation between the numbers of fungi and the numbers of bacteria plus actinomyces in soils where the influence of the reaction is no longer noticeable, suggests that the number of fungi in the soil obtained by the plate method is not merely a chance figure dependent on the accidental number of fungus spores, but has a real bearing on the abundance of fungi in the soil, although, as has been pointed out, the figure refers to the soil suspension and not to the soil itself. Also we must remember that in several of the decomposition experiments, such as those with cellulose and alfalfa seed meal in acid soil, there was in the later stages of the process an abundant, macroscopically visible fructification of the fungi. This is reflected in the constantly high counts of fungi in such cases (in opposition to those where the fungus counts, after having reached a maximum, soon decrease again, for instance with casein in acid soil and alfalfa seed meal in alkaline soil). In such cases the fungus counts become counts of spores and loose all significance as indexes of activity of the fungi, although of course the rise in numbers indicates that an active growth has taken place. In future work of this nature it would probably prove of great value to combine decomposition experiments with the method of McLennan (32) for distinguishing between fungus spores and mycelium in the soil.

SUMMARY

A study has been made of the fungus flora of 100 Danish soils of different types and of reactions varying from pH 3.34 to 8.35.

Microscopical examination showed the largest amounts of mycelium in acid soils rich in organic matter.

"Direct isolation" yielded mainly Trichodermae from forest, moor, and heath soils, and mainly Mucoraceae from field, garden, and salt marsh soils.

Plate counts showed numbers of fungi ranging from 24,300 to 46,000 to a gram of soil. Most common were the genera *Mucor*, *Zygorhynchus*, *Absidia*, a sterile form resembling *Cunninghamella elegans*, *Penicillium*, *Trichoderma*, and *Fusarium*. Besides these a number of genera of less constant occurrence were found. The Aspergilli were found only sporadically in ordinary soils but very abundantly in hot greenhouse soils. The genera *Fusarium* and *Phoma* were characteristic of cultivated soils.

The numbers of fungi showed no clear relationship to the type of soil, except that very heavy clay soils were poor in fungi; and no relation to the soil reaction was found. The numbers of bacteria plus actinomyces increased, generally,

with increasing pH values up to pH above 6, but the correlation between these numbers and the hydrogen-ion concentration was not very close, the correlation coefficient amounting to only −0.32. The ratio of fungi to bacteria plus actinomyces varied from 0.87 to 0.0026 and showed a very close correlation to the hydrogen-ion concentration, viz., a correlation coefficient of 0.82 for the whole set of data. From pH 6 and upwards this correlation did not exist, but it became manifest in the pH interval 5–6. A corresponding coefficient of very nearly the same numerical value could be calculated from the data of Waksman.

Addition of lime to acid soils did not markedly depress the numbers of fungi, but stimulated the bacteria and actinomyces greatly.

The resistance of fungi to acidity varies considerably, some species growing at pH 1.5, others being checked at pH 3.7–4.2. Only a few of them show a distinct optimum at acid reaction. The pH interval of 5.0–6.0 is critical for the majority of the ordinary soil bacteria, and most soil actinomyces are checked in their growth at pH 4.4–5.

Fertilization, especially with farmyard manure, increases the number of fungi in the soil, as well as those of bacteria and actinomyces. In soils of pH above 6 there is a significant positive correlation between the numbers of fungi and of bacteria plus actinomyces.

These results indicate that the actual abundance of fungi depends on many factors, among others the food supply, but the ratio of fungi to bacteria plus actinomyces seems dependent on little except the hydrogen-ion concentration.

Addition of dextrose to soil stimulated the fungi greatly in acid soil, but little or not at all in neutral or alkaline soil. The bacteria were affected reversely. Also here the difference appeared in the pH interval 5.1 to 5.9. The sugar appeared to be equally rapidly decomposed in acid and alkaline soil.

Most fungi except the Mucoraceae were capable of decomposing cellulose. Addition of cellulose gave rise to an abundant development of fungi in both acid and alkaline soil. Penicillia and Trichodermae prevailed in the acid soil, *Mycogone nigra* and *Coccospora agricola* in the alkaline soil. The fungi of the former group were more resistant to acidity and decomposed cellulose more rapidly in acid soil, whereas the reverse was true of those of the latter group.

Addition of casein gave rise to an abundant development of fungi (especially of Penicillia and Mucoraceae) in acid soil but not in alkaline soil, except when cellulose was also added.

Alfalfa seed meal caused an abundant development of Penicillia and Mucoraceae in acid soil, and a somewhat more limited growth of Mucoraceae and Fusaria in neutral to alkaline soil.

The proteolytic power of the soil fungi varied considerably according to the experimental conditions, but would in several instances rival or exceed that of *Bacillus mycoides* and *Actinomyces griseus*. The most strongly proteolytic fungi were not necessarily those appearing to grow most actively when protein was added to the soil.

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